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CLONAL FORESTRY:

IT'S IMPACT ON TREE IMPROVEMENT AND OUR FUTURE FORESTS

LA FORESTERIE CLONALE:

SON IMPACT SUR L'AMÉLIORATION GÉNÉTIQUE DES ARBRES ET NOTRE FORÊT FUTURE

WORKSHOPS

1. PRODUCTION AND UTILIZATION OF GENETICALLY IMPROVED SEED
2. ISOENZYMES IN TREE IMPROVEMENT
3. NORTH AMERICAN QUANTITATIVE FOREST GENETICS GROUP MEETING

ATELIERS DE TRAVAIL

1. PRODUCTION ET UTILISATION DES SEMENCES DE QUALITÉ GÉNÉTIQUE AMÉLIORÉE
2. ISOENZYMES EN AMÉLIORATION GÉNÉTIQUE DES ARBRES
3. RÉUNION DU "NORTH AMERICAN QUANTITATIVE FOREST GENETICS GROUP"

Canadian Tree Improvement Association / Association Canadienne pour L'amélioration des Arbres

TORONTO 1983

PROCEEDINGS

NINETEENTH MEETING
PART 2

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DIX-NEUVIÈME CONFÉRENCE
2e PARTIE

EDITORS / RÉDACTEURS
L. ZSUFFA
R.M. RAUTER
C.W. YEATMAN

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DE LA

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DE

L'ASSOCIATION CANADIENNE POUR

L'AMÉLIORATION DES ARBRES

2e PARTIE:

COLLOQUE SUR

LA FORESTERIE CLONALE: SON IMPACT SUR L'AMÉLIORATION GÉNÉTIQUE DES ARBRES ET NOTRE FORÊT FUTURE

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1985

PROCEEDINGS OF THE NINETEENTH MEETING OF
THE CANADIAN TREE IMPROVEMENT ASSOCIATION

With the compliments of the Association

Enquiries may be addressed to the authors or to Mr. J.F. Coles, Executive Secretary, C.T.I.A./A.C.A.A., Room 147, Johnston Hall, University of Guelph, Guelph, Ontario, N1G 2W1.

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The Twentieth Meeting of the Association will be held in Quebec, Quebec, August 19-23, 1985. Speakers will be invited to address the topic of "Accelerated Genetic Gains through New Technologies". Canadian and foreign visitors are welcome. Further information concerning the 20th Meeting should be addressed to: Dr. Armand Corriveau, Centre de Recherches forestières du Laurentides, C.P. 3800, Saint-Foy, Québec. G1V 4C7.

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La vingtième conférence de l'association aura lieu à Québec (Québec) du 19 au 23 août 1985. Des orateurs seront invités à s'adresser au sujet de "l'accelération du développement de la génétique grâce à la nouvelle technologie". Tous sont les bienvenus. Pour d'autres renseignements concernant la vigtième conférence, s'adresser à: M. Armand Corriveau, Ph.D., Centre de recherches forestières des Laurentides, B.P. 3800, Sainte-Foy (Québec) G1V 4C7.

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On behalf of the Canadian Tree Improvement Association, I gratefully acknowledge the Faculty of Forestry, University of Toronto, and the Ontario Forest Industries Aassociation for their support towards the icebreaker, banquet and invited speakers at the Nineteenth Biennial Meeting.

We are grateful to the Ontario Ministry of Natural Resources for providing the competent assistance of Louis Zsuffa (program), Jim Hood (local arrangements) and support staff Brad Graham, Dan McKenney, and Celia Graham.

The Association is also indebted to Sam Foster of the North American Quantitative Forest Genetics Groups, to Bill Cheliak and George Buchert for their organization of the isozyme workshop, and to Ben Wang and Doug Skeates of the Tree Seed Working Group for enhancing the C.T.I.A. conference with their annual meeting and workshop, respectively.

R.M. Rauter, Chairman

REMERCIEMENTS

De la part de l'Association canadienne pour l'amélioration des arbres je suis très reconnaissant à la Faculté de Forestérie à l'Université de Toronto, et à la "Ontario Forest Industries Association" d'avoir participé au "bris-glace", au banquet et aux discours au dix-neuvième conférence biennal.

Nous témoignons de la gratitude pour l'excellent appui de Louis Zsuffa (programme), Jim Hood (arrangements locaux), et Brad Graham, Dan McKenney et Celila Graham (auxiliaires) du ministère des Ressources naturelles de l'Ontario.

L'association sait gré à Sam Foster des "North American Quantitative Forest Genetics Groups", à Bill Cheliak et George Buchert d'avoir organisé l'atelier sur les isoenzymes, et à Ben Wang et Doug Skeates du Groupe de travail sur les semences forestières d'avoir contribué au conférence de l'A.C.A.A. en tant que hôtes du conférence annuel et de l'atelier.

R.M. Rauter, Président

Dr. V. Nordin, Dean of the Faculty of Forestry, University of Toronto and Mr. W.T. Foster, Deputy Minister, Ontario Ministry of Natural Resources, presents Dr. C. Heimburger with a plaque in recognition of his outstanding contributions to the knowledge and advancement of forest genetics and tree breeding.

M. V. Nordin, doyen de la faculté de foresterie à l'Université de Toronto et M. W.T. Foster, adjoint ministre, ministère des Ressources naturelles de l'Ontario donnent une plaque à M. C. Heimburger en témoignage de reconnaissance de ses excellentes contributions aux connaissances sur l'amélioration génétique des arbres et donc au développement de celle-là.

APPRECIATION/RECONNAISSANCE

DR. CARL C. HEIMBURGER

The Canadian Tree Improvement Association, the Faculty of Forestry, University of Toronto, and the Ontario Ministry of Natural Resources were proud to extend a special honour to Dr. Carl C. Heimburger at its 19th Biennial Meeting. Dr. Heimburger was presented with a diploma certificate and a special plaque in recognition of his outstanding contributions to the knowledge and advancement of forest genetics and tree breeing.

It was through Dr. Heimburger's foresight and enthusiams that tree breeding got its foundation in Canada in the 1930's. Dr. Heimburger continued to make historical progress in tree breeding, conducting most of his work in Ontario at the former Southern Research Centre in Maple.

Dr. Heimburger's contributions are acknowledged in Canada and throughout the world. He is particularly noted for his work in poplar breeding and white pine hybridization.

M. CARL C. HEIMBURGER

L'Asscciation canadienne pour l'amélioration des arbres, la Faculté de Foresterie à l'Université de Toronto, et le ministère des Ressources naturelles de l'Ontario ont eu le grand plaisir de rendre honneur à M. Carl C. Heimburger au dix-neuvième conférence biennial. On a donné à M. Heimburger un diplôme et une plaque spéciale pour témoigner de notre reconnaissance de ces contributions extraordinaires aux connaissances sur l'amélioration génétique des arbres, et à son développement.

C'était grâce à la prévoyance et au enthousiasme de M. Heimburger que les canadiens ont commencé à s'intéresser à l'amélioration des arbres dans les années 30. M. Heimburger a continué à réaliser des progrès historiques en ce qui concerne l'amélioration des arbres; il fait la plupart de son travail à Maple, en Ontario, à l'ancien Centre de recherche du Sud.

Les contributions de M. Heimburger sont reconnues au Canada et à travers le monde. Ses travaux sur l'amélioration des peupliers et sur l'hybridation des pins blancs sont les mieux connus.

C.T.I.A. EXECUTIVE AND GUEST SPEAKERS

L'EXÉCUTIF DE L'A.C.A.A. ET LES ORATEURS INVITÉS

Dr. L. Zsuffa, Dr. A. Franclet, Dr. G. Vallée, Dr. K. Ohba, Dr. J. Bonga
Mr. J. Hood, Dr. C. Heimburger, Dr. W. Libby, Miss R.M. Rauter,
Dr. D. Fowler, Dr. B. Dancik, Dr. J. Kleinschmit.

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KEYNOTE ADDRESS

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KEYNOTE ADDRESS

POTENTIAL OF CLONAL FORESTRY

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Keywords: Deployment strategy, Diversity, Maturation, Selection, Silviculture

INTRODUCTION

To most people, the word "clone" is not a neutral word.

To some, the images are wonderful and even magical, full of the promises of modern molecular biology.

To others, the word is ominous, leading inexorably to visions of that worst of all monocultures: large, dreary, and biologically unstable monoclonal plantations.

RECENT HISTORY

In Canada, clonal forestry was pioneered during the 1940's by Carl Heimburger, largely with poplars. Here and in most other parts of the world, the idea of a clonal forest of conifers was rarely addressed until about a decade ago. Except for a few species, most of which were hardwoods, the biological problems kept clonal forestry from serious consideration. Among those biological problems, now largely solved or understood, are maturation state, cutting environment, cutting condition, problems associated with other ways of cloning, and survival and growth of propagules.

Maturation State

It was logical to select outstanding trees as cutting donors for clonal trials and for use in forest plantations. Trees of at least half rotation age seemed likely to provide better information for selection than younger trees; thus mature trees were generally used as sources of cuttings. It only took a few rooting trials to learn that cuttings from mature trees did not root very well. Furthermore, those cuttings that did root didn't grow very well. But few of those hopeful early cloners realized that the maturation state of their cuttings was the problem. Even fewer thought that maturation state could be controlled, or better yet, manipulated. It now seems clear that maturation can be slowed, perhaps arrested, and maybe even reversed.

Cutting Environment

We have learned much about the physical environment of detached cuttings, and we now can modify that environment so that cuttings are maintained in a healthy condition prior to their rooting. We know (with less certainty) that many elements of that environment, such as bottom and top temperature, also affect the rooting event.

Cutting Condition

We have learned much about the physiological states of cuttings, and we have particularly gained experience with various plant-growh regulators that promote or otherwise affect rooting.

Diseases

We are beginning to learn about various important pathosystems and their interactions in various rooting environments. The low rooting percentages common in many early experiments were not so much rooting failures as pathogen successes.

Other Ways of Cloning

Grafting was occasionally used in order to produce genetic uniformity for various experiments, and even for large-scale propagation. Cost, stock-scion incompatibility, and variable rootstock effects have largely ruled out these uses. Grafting is still appropriately and effectively used for seed-orchards, breeding-orchards, gene archives, and in some urban-forestry operations. Organ- and callus-culture techniques have progressed from exotic science to become increasingly reliable alternatives to the more classical rooting of cuttings. Encapsulated embryoids may soon be the method of choice.

Survival and Growth

Much of the early skepticism with regard to the practical usefulness of rooted cuttings (stecklings) was based on their poor survival and early growth in field conditions. We are gaining experience with the after-care of newly-independent stecklings. Increasingly, we can produce healthy stecklings that survive as well and grow as well as the best nursery-produced seedlings.

In short, while we still need to know the answers to a lot of biological questions to do clonal forestry well, these biological questions are no longer of a disqualifying nature, such that they block further consideration. We can now begin to think seriously about other kinds of questions. Questions concerning the economics of clonal forestry, and policy questions related to clonal forestry, are and will be important. However, in this paper, I am going to focus on opportunities and strategies.

DO WE NEED A WHOLE NEW DESIGN?

A favourite analogy has to do with the strategies followed by three major U.S. aircraft builders following World War II, with respect to the civilian passenger market[1]. In the 1930's, Lockheed was producing their two-engine "10" and "14"' series, and Boeing came out with their "247" in 1933. Beginning in 1936, Douglas had the DC-3, which for years was thought by many to be the best and most reliable airplane in service. The four-engine Boeing Stratoliner appeared in 1940, and then further civilian development paused for World War II. Near the end of that war, Allied air crews got their first looks at German jet aircraft.

Following the war, piston engines continued to be used on civilian airliners for more than a decade. Douglas' wartime four-engine DC-4 was soon replaced by its popular DC-6. Boeing upgraded its Stratoliner to a Stratocruiser. In the early and mid-1950's, Douglas followed the DC-6 with a series of DC-7's, culminating in the large, long-range DC-7C. Lockheed added two engines and a tail to evolve its 10A Lodestar to its Constellation, and then to its wonderful Superconstellation. These 1950's models were about as good as big piston-engine airliners ever got.

Responding to much pressure and direction from Eastern Air Lines' Captain Eddie Rickenbacker, in 1959 Lockheed produced the Electra, the first U.S. civilian airliner with jet engines. The Electra looked like a fat DC-7, with turbojets mounted where the piston engines used to be. (Perhaps that was comforting to those who were used to the DC-7, and thought it was wonderful.)

Meanwhile, Boeing had been gaining experience with a jet-powered military tanker, which was designed to take advantage of the jet and to meet its requirements. By 1959, they had modified it for civilian passenger use, and entered that market with the 707. A year later, Douglas brought out the DC-8. It looked a lot like the 707. Since 1960, no new major long-haul airliner has looked anything like the wonderful and successful DC-3 or DC-7, and most have looked rather like the 707.

I now suggest, perhaps inappropriately, that those forestry enterprises that attempt to use stecklings or culture-origin plantlets or embryoids in a forestry system designed for seedlings will be creating something about as successful as the Electra. Further, those that persist with the tried and true seedling as the propagule of choice will be about as competitive as a 1983 airliner salesman with a lot full of new DC-7's.

But, what are the probable design changes that will produce 707-like clonal forestry?

---

[1] The material on aircraft in this section, of which I know little, is from Wigton, D.C., 1963, From Jenny to Jet, Floyd Cramer, Los Angeles

BE CAUTIOUS WITH FANTASIES

I opened this talk speaking of promises of modern molecular biology and fears of unstable monocultures. I believe both are largely fantasies.

Molecular Biology Fantasies

As exciting as molecular biology is, there are several reasons that led me to believe that it will not soon provide much to forestry that is radically new. The first and most obvious reason is its expense. But clever people may solve the problems of expense.

A more serious problem is that plants produced by the more radical techniques of molecular biology will be totally new. And being totally new, they must be very carefully tested. Thus, the promise of faster entry of new clones to production forestry through molecular biology appears to me to be a fantasy, and perhaps a dangerous one. Because propagules for forest plantations must successfully occupy a variety of sites for very long periods, the tests of radically different trees should be conservative;i.e., they should be conducted on many sites for many years, exposing such trees to both the foreseen and unforeseen conditions that occur on operational plantation sites. It is the need for this conservative and long-term testing that will increase both the time and the expense of entering radically-engineered new clones.

In contrast, one may use clones whose histories include conventional selection from populations known to be adapted to plantation sites, followed by breeding, clonal testing, and further selection. Such clones are at least as reliable as seedlings from those same populations. They may be rapidly entered in large-scale use, with considerable assurance that most of them will not fail. This is not to say that radical new clones will never be used. It merely suggests that their use will (should) be neither soon nor cheap.

The safest technique and thus probably the most useful genetic-engineering technique for forestry, will be genetic surgery..... i.e., the insertion of single desirable genes (operons) into clones that are already outstanding in most other respects. At present, this technique is not operational with forest-tree species. Furthermore, few sufficiently outstanding recipient clones of forest trees are available..... perhaps the poplar clone I-214 is one such.

Large Monoclonal Fantasies

The fear of inexorable, large, unstable, monoclonal plantations is, I think, fear of a fantasy. Large monoclonal plantations are neither necessary nor likely components of clonal forestry. If they occur at all, they are best viewed as rather nasty management errors, and such errors may be avoided by education and/or regulation.

Well, none of the above leads obviously to our 707-like forestry.

SO WHAT IS TO BE REDESIGNED?

Production Options

With greater per-tree (or per-unit-area) productivity being acheived by genetic means, managers have the option of reducing the amount of land managed for harvest, or of increasing the total production on the previous land base. These options occur with any form of successful tree-improvement, but clonal forestry appears to offer the greatest genetic leverage.

Orchards

Classical tree-improvement has typically relied on seed-orchards to produce select stock at production levels. Seed-orchards also feature sexual recombination of the select genotypes, a mixed blessing at best. As clonal programs come on-line, seed-orchards will be replaced by much smaller and less demanding breeding-orchards, to satisfy the need for continued advanced breeding. It is appropriate to emphasize here that continued breeding will be as essential a component of clonal forestry as it is for classical tree-improvement. But for most clonal forestry, production-level multiplication of clones will be done in hedge-orchards, or by using young stecklings still in the nursery as donors, or by in-vitro propagation.

Returning to the airline analogy, piston-engine airliners still serve on local low-volume runs. Here the analogy breaks down. Seed-orchards are sensitive to economies of scale, and one can rarely justify a seed-orchard for local low volume demand. (In contrast, a relatively few hedges will satisfy such a special demand, and they can easily be included in a larger hedge-orchard.) Seed-orchards are site-demanding, can be labour-demanding when cones ripen, should be free of pollen contamination, and can have other annoying management problems. You may wish to take pictures of these wonderful old components of seedling-based forestry. If so, do it soon, for they will disappear soon after reliable clonal programs become available.

Nurseries

If clonal forestry employs plantlets or stecklings, nurseries will be substantially redesigned in their early operations and little affected in their later ones. Seed-handling equipment will be replaced by mechanized methods of sticking cuttings. If plantlets are employed, we will need new techniques and facilities to acclimatize the delicate plantlets from culture conditions to harsher field conditions. Compared to a seedling, a recently rooted steckling needs more attention to the early form of its roots, using either containers or root pruning for this purpose.

Once the stecklings or plantlets are well established, husbanding of their continued growth and their preparation for lifting will be very similar to what is (or should be) done for seedlings. If it becomes operationally possible to encapsulate cloned embryoids, then

nurseries may handle these very much like they handle seeds today. However, in order to take full advantage of clonal forestry, clonal identities should be maintained at least until assignment of the propagules to a plantation site, and thus record-keeping at clonal nurseries will have greater detail.

Finally, when all clones in production use are of high genetic quality, culling percentages should be greatly reduced and the efficiency of nursery management will thereby be improved.

Reciprocal Effects

Much of what used to be known as "maternal effects" is turning out to be due to DNA contained in mitochondria and chloroplasts, exclusively (or largely) inherited from the mother. Much hard evidence is accumulating from molecular biology that this mitochondrial and chloroplast DNA is of importance and is variable. Softer evidence has been bothering us quantitative-genetic types for some time, in that open-pollinated families told us more about the female's performance as a mother than we thought they should.

For some time, I've been recommending single-pair matings as an efficient and cost-effective mating design for long-term (many-generation) breeding. And it is, at least for nuclear genes that are largely additive in their effects. But, if most or all mitochondrial and chloroplast DNA is inherited from the mother, as much as half of the variation in these genes will be lost in the first generation of single-pair matings, and more may be subsequently lost depending on the assignment of male or female roles to sibs. This requires some rethinking, no matter whether a clonal or seed-orchard option follows the breeding. But note that clones can capture this variability better than can an open-pollinated seed-orchard.

Silviculture

As with other tree-improvement options that increase the growth and value of plantations, clonal forestry will make various silvicultural manipulations more possible and more effective. Because clones will provide the greatest predictability, the timing and effectiveness of particular silvicultural treatments can be best optimized with known sets of clones. The nature of the treatments prescribed may also vary substantially, depending on the particular clones in a plantation.

Site Characterization

The clonal option allows the selection of sets of clones adapted to particular sites. Thus, with clonal forestry, there will be much greater attention to characterization of sites and subsites when planning plantations, prior to ordering the clonal mixtures for them.

Deployment Strategies

As we learn more about general theory and as we accumulate specific details about our best clones, the decisions we make about the numbers and arrangements of these clones may have large effects on both the appearance and the scheduling of our forest stands. The two deployment strategies being frequently debated are "widespread intimately mixed plantations" vs "mosaics of monoclonal stands".

Early in a program, it will be prudent to deploy large numbers of local clones to any given plantation site, and it will also be prudent to plant them in intimate mixture. Those that are well adapted will sort themselves out from those that are not, and, with close initial spacing, sites should thus remain fully occupied.

Later, both biological and management arguments may lead us to favour mosaics of small monoclonal plantings. For this option, we will probably use relatively few clones in each region. Ideally, these clones will be well known (i.e. thoroughly tested) and biologically dissimilar to each other.

When we learn even more, we may be able to program particular neighbour sequences during planting, such that adjacent clones make complementary demands on the site, or such that a thinning sequence is pre-programmed by clone and position. It may be that particular clones of two or more species can coexist in intimate mixture, even though random members of the same two or more species do not mix well.

THE TITLE OF THIS PAPER

I think that there are at least 16 major potential advantages to clonal forestry.

One advantage is the short-term ability to capture a greater proportion of the additive genetic variation, a greater proportion of the variation in chloroplast and mitochondrial genes, and a much greater proportion of the nonadditive genetic variation, than can be done using conventional seed-orchards. However, note that over several generations of breeding, additive nuclear alleles and favorable chloroplast and mitochondrial gene complexes will be accumulated, and the additional gain possible by cloning will be relatively reduced when compared to the total accumulated gain. Put another way, long-term genetic advances will depend on breeding; clonal techniques will add a gain increment in each generation, by locating and propagating those outstanding individuals that have unusually favourable combinations of genes.

A 2nd potential advantage is the ability to provide adapted (and probably selected) clones for unusual sites. Such sites are not well served by families selected for the more common sites, and these unusual sites do not require regeneration in numbers to justify their own conventional seed-orchards.

A 3rd is the elimination of all inbreds (including selfs) from production plantations. For most species. open-pollinated seed-orchards may produce an unacceptable percentages of selfs. Clones derived from pedigreed control-pollination crosses can be 100% non-inbred. This is particularly useful in species that exhibit serious inbreeding depression in plantations, but whose inbreds in good nursery conditions have high germination percentages, high viability, and early growth above cull levels.

A 4th is the mass production of valuable but expensive genotypes. These include various kinds of hybrids, the products of various genetic-engineering techniques, and pedigreed control-cross seedlings.

A 5th is the identification of broadly-adapted and/or highly interactive clones, with deployment of the latter then being restricted to those sites on which they do well.

A 6th is the ability to use maturation states other than juvenile. In at least some species, late juvenile or even early adolescent maturation states of their propagules at the time of planting confer substantial advantages with respect to subsequent stem-form and resistance to at least some important pests.

A 7th is the possibility of using "correlation breakers". Multiple-trait index gains attainable by sexual reproduction, on average, are constrained by genetic correlations between the indexed characteristics. However, these correlations are rarely 1.0, and unusual "correlation breakers" can be found by clonal testing.

An 8th allows us to shift biomass allocation in our plantations from cones and pollen to bole wood. While there is no convincing evidence that juvenile growth is negatively correlated to seed and pollen production, it makes sense that heavy commitments to reproductive ograns are probably at the expense of something else after reproductive maturity is achieved.

A 9th is the knowledge and experience that accumulates about outstanding successful clones. (We must avoid the mistake of using too high a proportion of too few such clones.) These well-known clones will probably remain more valuable than new clones or seedlings from the breeding program, which are genetically better but less known than the current clones. This may sound like a disadvantage with respect to gain per unit time, but the advantages of managing a well-understood set of clones are substantial.

A 10th is the control of genetic diversity in production plantations. Genetic diversity may be more precisely prescribed using sets of pedigreed clones than using the continuum of unique genotypes produced in the wild or in seed-orchards. Here, the goal is not merely plantations that are as safe as those using seedlings, but plantations that are significantly safer as a result of wise and informed deployment of clones.

An 11th, related to the 2nd and 10th, is the ability to improve and thus effectively use a greater number of species. Two elements of clonal forestry contribute to this. One, noted above, is the possibility of mixing compatible clones of species that do not typically mix well. The second is that, in a clonal program, the production of individual clones is not as sensitive to economies of scale as is classical seed-orchard production of improved seedlings. Thus, while it might be economically possible to genetically improve (and thus effectively use)only two or three species in a region by classical seed-orchard techniques, clonal selection and propagation may allow deployment of genetically superior clones from a much larger number of species.

A 12th is the greater simplicity of managing hedge-orchards (or other donor options) than of managing seed-orchards. In addition, the unit of management will be the individual clone, rather than the general seed-orchard. This removes several restrictions (such as putting clones that should not interbreed in the same hedge-orchard), and increases flexibility (each clone is individually deployable to different plantations).

This latter allows a 13th advantage, namely specific and probably optimal deployment of sets of clones from the available list of known clones to each plantation or even to each subsite of a plantation. It is likely that no two plantations will have exactly the same set of clones. In contrast, most seed-orchards produce the same general product for all their client areas.

A 14th, true in most species, is the shorter time between selection and production, compared to seed orchards.

A 15th, discussed above, is the ability to program planting sequences, such that productivity is increased by reducing negative competitive interactions, and/or such that thinning and other activites may be planned by clone and position.

The 16th concerns selection philosophy. In most seed-orchard programs, in an attempt to quickly use the very best trees from a large number of candidates, one usually selects a very few of them. These few selected individuals become the parents of extensive production plantations. In contrast, most clonal programs begin with many clones and selection is then incremental. As different characteristics develop at each round of evaluation during the tests, new increments of the poorer clones are identified and discontinued. The remaining clones contribute ever larger proportions to the clonal production plantations. This incrementally reduced proportion of clones in production is both increasingly better genetically and increasingly better known, and thus is increasingly reliable. This latter strategy seems much more in keeping with the conservative selection philosophy appropriate to a long-term crop such as forest trees.

THE BEGINNINGS OF CLONAL FORESTRY

One condition that is necessary is that the cloned ramets must develop into satisfactory trees. Given that, clonal forestry may be adopted when sufficient additional conditions are present. For example, clonal forestry has been pioneered with such species as cottonwoods, willow and tsugi, whose rooted cuttings cost little more (or even less) than seedlings.

Other sufficient conditions are that unit values are high and rotations short. The Christmas-tree industry is thus characterized, and it is beginning to replace seedlings with expensive but more reliable clones.

When tree values or maintenance costs are very high, very expensive clones (that produce unusually valued trees or that avoid costly problems) can be justified. The emerging field of urban forestry is rapidly adopting clonal propagation of select trees.

When trees are planted at wide spacings, as in agroforestry, clonal reliability is highly desirable. Thus, agroforestry will probably adopt the clonal option even though per-plant costs remain higher than those for seedlings, and values are not unusually high.

For more general wood-and-fiber forestry, two forces appear to be at work. On the one hand, stumpage values are expected to continue to outpace inflation; and land, labour, and transportation costs will make high unit-area productivity increasingly desirable. On the other hand, technical advances in producing clonal propagules are eliminating maturation-related problems, and are reducing unit costs. The joint effects of these forces will be to shift conventional forestry to clonal forestry, species by species and region by region, as values and costs become first favourable, and then compelling.

CONCLUSIONS

Forestry, compared to agriculture, has been very late in domesticating the organisms it depends on. Clonal forestry provides a major opportunity to close some of that gap. It seems inevitable that we will adopt a more aggressive management of the forest resource; the only thing likely to stop this intensification is the failure of civilization. But inevitable or not, it is exciting to be in on the development and expansion of clonal forestry. However, let me suggest that our excitement and enthusiasm should be tempered by the careful conservatism appropriate to husbanding a long-term resource, and that we should seek wisdom in instituting not only the techniques but the policies appropriate to clonal forestry.

Finally, I'm pleased to note that this first major North American conference featuring clonal forestry is being held in Ontario, where so much of the pioneering has been and is being done.

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SESSION I

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CONCEPTS AND EXPERIENCES IN CLONAL PLANTATIONS OF HARDWOODS

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ABSTRACT

Clonal silviculture has only evolved in poplars and willows. Therefore, consideration of the concepts and experiences in clonal plantations is restricted to these two genera.

Monoclonal planting is common in all forms of poplar and willow plantations because of ease of propagation by rooting of cuttings, advantages of growing and utilizing plantations of uniform trees, and limited availability of outstanding desired clones. Most of the plantings over large areas and in different countries are with very few clones.

Multiclonal plantations are a desirable alternative to existing monoclonal plantations in poplars and willows. The concept of "multiclonal varieties" in individual tree mixtures may be feasible for forest stand management. Mosaics of monoclonal blocks that are relatively small and similar in size in which clones are matched to soils, plantation systems and production objectives seem to be the only reasonable choice for intensively managed plantations.

Stock for multiclonal management has yet to be developed. However, active breeding projects in poplars and willows have the capacity for producing such stock. The prospects for similar development in other hardwood species are more distant.

RÉSUMÉ

La sylviculture des clones ne s'est développée que pour les peupliers et les saules. Par conséquent, il ne saurait être question que de la théorie et que des expériences touchant la plantation de clones de ces deux genres.

L'aménagement monoclonal est répandu dans les plantations de peupliers et de saules de toutes formes, en raison de la facilité de multiplication de ces espèces par racinage de boutures, des avatages de cultiver et d'utiliser des arbres uniformes et de la disponibilité limitée des clones exceptionnels voulus. La plupart des plantations sur de vastes territoires et dans différents pays sont établies avec très peu de clones.

Les plantations multiclonales représentent une solution de rechange souhaitable aux plantations monoclonales existantes de peupliers et de saules. L'idée d'avoir des "variétés multiclonales" dans des mélanges d'arbres individuels pourrait convenir à la gestion des peuplements forestiers. Des mosaïques de blocs monoclonaux de dimensions similaires, mais relativement petites, où les clones seraient choisis en fonction du sol, du système de plantation et des objectifs de production semblent la seule solution raisonnable pour les plantations en gestion intensive.

Le matériel nécessaire pour l'aménagement multiclonal n'est pas encore disponible. Toutefois, des travaux en cours dans le domaine de l'amélioration des peupliers et des saules pourraient produire ce matériel. Par ailleurs, les perspectives de réalisations similaires avec d'autres espèces feuillues sont plus éloignées.

INTRODUCTION

Clones of hardwood (broadleaved) tree species (Angiospermae Brongn.) have been used as ornamental and fruit producing varieties for many centuries. Hillier's manual of trees and shrubs (1974) lists more than 2,000 such clonal varieties, some dating back to the beginning of known human history. Some of these have been traditionally reproduced by grafting, while others have been reproduced by rooting of cuttings. Poplars (Populus L.) and willows (Salix L.), fall within this latter category. They are well-known for their ease of propagation by rooting of cuttings, and are, therefore, commonly used.

Ease of propagation by rooting of cuttings has significantly contributed to the distribution and use of hardwood clones. While varieties propagated by grafting remained restricted as high-value, individual trees for ornamental and fruit-producing purposes, other species which could be easily propagated by cuttings were inexpensively spread in large numbers. Such clonal material was also used in multi-tree, forest-type plantations for timber production. In addition to ease of rooting, the determining factors for such use were the utility of the wood, facility of plantation establishment, and adaptability to a variety of sites.

Poplars and willows conform well to these criteria and are valuable for timber production. Therefore, their clonal culture is the most widely spread of any hardwood species in a temperate climatic zone. Although some other hardwoods, such as Platanus L., have the potential for vegetative propagation, the concept of clonal silviculture evolved only in the case of poplars and willows as the result of a unique combination of economic demand, site requirements and ease of reproduction. Consideration of the concepts and experiences in clonal plantations of hardwoods in this paper is, therefore, restricted to these two genera.

HISTORICAL NOTES

Poplars are interspersed throughout all forests of the temperate and colder regions of the northern hemisphere. Willows have a wider natural distribution and also occur in the southern hemisphere. They grow in clusters and rarely expand into forests to a great extent. Many species of both genera are typical occupants of recent alluvial deposits.

In the International Poplar Commission's book on poplars and willows (FAO, 1979), the spread of cultivation of these species is described as follows: "when the forests of the plains of the Old World were cleared and converted to agriculture, many parts of the land were left aside because no worthwhile crops could be expected from them. However, it was there that poplars and willows were successful in establishing themselves. People came to look at these areas of natural growth to satisfy their needs for timber and fuelwood. Because of the value of the trees and the proven ease of propagating them by cuttings, people started planting them near their houses and around their fields to furnish wood and provide greenery and shade".

In Mid-Asia, the Near-East and around the edges of the Mediterranean, Salicaceae have been closely associated with agriculture since antiquity. For many centuries, pollard willows have provided fuel and withes, while poplars provided timber and fuel. As well, poplar leaves were used as forage and litter for animals. Even today, from Kashmir to the Atlantic in the Old World and throughout the New World, poplars and willows mark the neighbourhoods of human habitations.

The oldest type of poplar forest culture evolved with locally available trees; almost everywhere, this was with various forms of Populus nigra L. (Zsuffa, 1974). However, the introduction of P. deltoides Marsh from North America into Europe during the seventeenth century resulted in natural hybrids (P. deltoides x nigra = P. canadensis Moench, syn. P. x euramericana (Dode) Guinier) which in time revolutionized the ideas about poplar cultivation. Gradually, this led to the use of poplar in all modern wood industries.

The extremely rapid growth and ease of propagation by cuttings fascinated poplar growers. Nurseries were established and their output was in great demand. It was clearly appreciated that these poplars, although of the same origin, were not alike, and people wanted to know which clones were best suited to their needs. For planting, culture, and protection against pests, the empirical approach was the only answer.

During the first years of this century, poplar cultivation was already widely spread in Europe with the support of industry. Voices began to be raised on all sides deploring the general state of disorder, the ignorance of tree planters, and the ravages caused by insects and diseases. Scientists began to take interest in poplar; in France, Dode (1905); in England, Henry (1914), in Italy, Jacometti (1933); in Germany, Wettstein (1933); in the United States, Schreiner and Stout (1934); and in the Netherlands, Houtzagers (1937).

These initiatives were brought together in 1947 with the founding ofthe International Poplar Commission under the aegis of the Food and Agriculture Organization (FAO). The efforts of this Commission resulted in considerable progress in the knowledge of poplar types and their cultivation, and led to important agreements in nomenclature, registration of clones, varietal control, and the exchange of cuttings. The Commission, of which Canada is a member, is still very active, and is now also concerned with the cultivation and utilization of Salicaceae.

TYPES OF POPLAR AND WILLOW PLANTATIONS

The two main forms of plantations are in stands (blocks) and rows. A variety of each is practised in different countries depending on needs, tradition, tree varieties, sites, and economies. The examples which follow will illustrate these practises and also facilitate the discussion on clonal concepts and experiences.

In stands, poplar is grown most often in medium spacings (3.0 m to 4.0 m) and harvested in twenty to thirty year rotations. Rooted stock is used for planting, and some form of site preparation and initial tending is practised. The stands are at times pruned and thinned depending on growth vigour, tree variety, and the desired tree size and quality at harvesting. Such forms of plantations are found, for example, in Scandinavia and parts of mid- and eastern Europe with aspen (P. tremula L.) seedlings and its hybrid varieties (aspen does not propagate from cuttings), and throughout mid-Europe and in parts of North America with Euramerican (P. x euramericana) poplar and various balsam poplar hybrid clones.

Another common form of poplar plantations uses widely spaced trees (5.0 to 8.0 m) and harvested in ten to fifteen year rotations. These are clonal plantations of Euramerican poplar, cottonwoods, and similar easy-to-propagate varieties. Large-size rooted stock is usually planted on well-prepared sites, and the plantations are well tended. An example of this is the well-known Italian poplar culture. It has spread, together with clones developed in Italy, throughout the world. Such poplar plantations, when established on productive agricultural soils, are associated with annual farm crops (corn, potatoes, etc.) for 2 to 4 years until the crops become shaded by fast growing poplars. In other cases, forage, such as alfalfa, is grown and grazed by cattle in widely spaced poplar plantations.

A traditional form of poplar cultivation is practised in Asia, the Mid East and North Africa. Fastigiate (pyramidal) poplar clones (usually of P. nigra and P. alba L. origin) are grown in very dense spacings (1.0 m) in ten to twenty year rotations. The trees are an important source of timber for rural construction, and fuel, and leafy branches are used for animal feed.

Another form of poplar plantation has been developed in North America. Unrooted cuttings of easy-to-propagate clones are used for medium density plantations, and the production goal is usually industrial

fibre. The planting sites are prepared by ploughing, and the plantations are tended by cultivation and weed control. The rotation age is approximately ten years, and the trees are occasionally regenerated from coppices.

Biomass plantations are a new concept in poplar and willow cultivation. These are very dense plantings (0.5 m to 1.5 m) of unrooted cuttings, harvested frequently (every 1 to 5 years), and subsequently regenerated by coppice growth. The blocks are monoclonal, and the trees only grow well on fertile sites and when properly tended. The whole above-ground portion of the trees, sometimes even the foliage, is harvested and used as a source of fibre, energy and food. A traditional form of willow plantations with a concept similar to biomass plantations are the osiers.

Willow stands are in many cases similar to poplar stands. However, dense plantings (3.0 m) are more commonly practiced, and tending is usually less frequent and extensive. Willow cultivation is widely spread in Europe along the Danube River, and in Argentina in the valley of the Parana River.

In row plantings, there is a variety of cultivation forms of poplars and willows with different cultivars. A common form of row plantation uses Euramerican poplar clones along ditches, roads, and farm boundaries. The spacing is usually wide (4.0 m to 10.0 m) and large, rooted stock is planted in single rows, or occasionally, in double rows. The trees are cut at twenty to forty years-of-age and replaced by new plantings. This form of row planting is very common throughout Europe. In some countries, such as parts of Holland, France and Italy, approximately 40 trees grow in such plantings on every hectare of farmland.

In the Mid East and North Africa, fastigiate poplars are often grown in single or multiple rows in dense (1.0 m to 2.0 m) arrangements. In the Soviet Union and Canada, poplars are an important component of windbreaks. In New Zealand, row plantations of poplar check soil erosion as a result of sheep and cattle grazing on hilly land.

A traditional concept of row plantations, practiced in many Old World countries, are the pollard willows. The crowns of these willows are cut back periodically to the trunk and the branches are used as fuel, withes and stakes.

CONCEPTS IN CLONAL PLANTATIONS

Monoclonal planting is common in all forms of poplar and willow plantations. This concept was applied almost automatically as the most reasonable and practical method of poplar and willow cultivation.

The reasons for, and practicality of, monoclonal poplar and willow culture are: ease of propagation by rooting of cuttings; the advantages of growing and utilizing plantations of uniform trees; large

variation within populations and families; and the occurrence of unusual, desired individual types. The ease of vegetative propagation alone, if dealing with relatively uniform populations and sibs containing many desired types, could have lead to multiclonal cultures. However, poplar species in general, and families of interspecific hybrid poplars in particular, are well-noted for large tree-to-tree variation. In fact P. nigra clonal selections of fastigiate forms, such as cvs. italica, thevestina and plantierensis, which have been propagated for centuries, represent unique or rare occurrences. Old selections of Euramerican poplars, such as cvs. serotina, regenerata and robusta, are also distinguished by particular features of form, growth and site adaptation. For this reason, and because of the relatively late start of planned poplar and willow breeding programs, the number of clones used in monoclonal plantations throughout the world has remained relatively small.

The International Poplar Commission has registered fifty-two clones and has eight more clones which are candidates for registration (FAO, 1983). Most of these clones are Euramerican poplar hybrids. The Commission's textbook on poplars and willows (FAO, 1979) describes five clones of P. nigra, twenty-two clones of P. deltoides (in use since 1970), forty-four clones of Euramerican poplar (about half of them in use since 1970), and twenty clones of balsam poplars and their hybrids (eight of which have been in use since 1970). Thus, the total number of poplar clones registered and in commercial use is less than one hundred.

The situation with willow clones is similar in that the total number of clones in commercial use does not exceed one hundred. However, many of these are osier willows (shrubs); few clones are actual trees.

While osiers and biomass (energy) plantations are always monoclonal, tree plantations of willows, especially of Salix alba L. along the Danube River, are occasionally multiclonal. This is because special selections were not made earlier within this species which is native to the flood plain of the river, and the plantations established on similar sites are extensively managed as forest stands.

Only a portion of registered poplar and willow clones are widely used in plantations. According to the reports of the National Poplar Commissions submitted to the 16th Session of the International Poplar Commission, the number of clones planted on a large scale is very small in most of the countries (Chardenon, 1980). In Hungary, three clones (P. x euramericana cvs. Robusta, I-214, and Marilandica) represent 81.0% of the plantations, in the Netherlands, Euramerican poplar clones Robusta and Zealand account for 60.0%, and in France, Robusta and I-214 account for 70.0% of the plantings. In the Republic of Korea, Euramerican poplar clones I-214 and I-476 are planted almost entirely on the plains, while seedlings of a single hybrid aspen family (P. alba x glandulosa) are planted in the hills and lower mountain ranges on a total of 428,000 ha. A few of the clones, such as I-214 and Robusta, have been very successful and are, therefore, widely planted in many countries. Fewer than ten clones represent well over 50.0% of all poplar plantations, the extent of which can be estimated at more than 1,500,000 ha worldwide.

The situation in willow plantations with regard to the number of clones registered and planted over large areas is similar to poplar. In England, a single clone (S. alba v. coerulea) has been planted and used for the production of cricket bats. In osiers, as well as in tree plantings, a handful of clones are favoured and used throughout the world.

Multiclonal concepts were introduced in poplar and willow culture in the 1960's in conjunction with the dawn of new ideas in plantation technology, spreading of plantations to different sites, and well-planned breeding programs. The ideas on integrated use of trees (the biomass concept), and new needs and possibilities in biomass utilization (such as for composite boards, energy, food) led to studies of matching clonal characteristics to production and utilization technology. These studies showed significant clonal variation and resulted in new clonal selections and diversification.

After the Second World War, poplar plantations spread to new and different sites, such as forest sites, upland sites, and different countries and climatic zones. Occasionally, traditional clones did not perform satisfactorily in such conditions and a search for new selections was initiated. At the same time, some of the well-established breeding programs, such as in Belgium, (Steenackers, 1970), Germany (Mohrdiek et al., 1979) and Holland (Koster, 1976), have come to fruition. In addition, new breeding programs were initiated by geneticists and specialists.

All this resulted in a variety of new breeding stock in the arboreta with new combinations of hybrids, and a large number of new clonal selections. However, although the need for diversification of clonal stock was realized and breeding efforts for this diversification were made, multiclonal concepts were only considered and developed in a few cases.

The two main concepts promoted for clonal mixtures were: (i) the use of mosaics of relatively small monoclonal blocks which were similar in size, and (b) the use of row-to-row or tree-to-tree type clonal mixtures.

The concept of mosaics of monoclonal blocks in poplar plantations was developed and implemented in Ontario, Canada (OMNR, 83). According to this concept, clones are carefully matched to site, plantation system and production goal. Sites are planted with several, rather than one or two, different clones. Monoclonal blocks are no larger than 5.0 ha in size. Each clone in production is continuously being tested for growth and pest resistance. As better clones become available from on-going breeding work, the poorer clones are removed from nursery propagation and planting. The number of clones in propagation and planting is more than fifty at any one time, and 5.0 to 10.0% of the clones in production are changed annually by phasing-out and new introductions.

The poplar plantation program in Ontario, as well as the breeding work in support of this program, has intensified since the late 1960's. The existing clones did not, in many cases, satisfy the needs,

and studies demonstrated the significant potential for new combinations of hybrids and clones (Zsuffa, 1976, 1979). Team-work, and cooperation between research, technology development and management led to the development and successful application of this multiclonal concept in Ontario.

The use of row-to-row and tree-to-tree type clonal mixtures was attempted in Holland and Germany. Kolster (1978) investigated the possibility of decreasing monoclonal risks by mixing two clones by rows in plantations established with five hundred to six hundred and twenty-five trees/ha. The results indicated that this method did not offer practical possibilities. The growth of one clone was suppressed by the other. As well, the loss in wood production by one clone was not compensated by the increased growth of the trees of the other clone. For this reason, the mixing of rows of different clones was discouraged. The same result was expected when individual trees of different clones were mixed.

The concept of such mixtures of individual trees of several clones, or "multiclonal varieties", was furthered by Weisgerber (1979) in WestGermany. With the spreading of poplar culture to forest land in that country, attention turned to aspen and balsam poplars which placed less demand on climate and soil than cottonwoods and black poplars (section Aigeiros Duby). While aspen stock is rarely clonal due to difficulties in rooting of cuttings, balsam poplar stock often occurs as clones. In 1972, three balsam poplar clones comprised 54.0% of the market, and the proportion of balsam poplars to all poplar stock produced grew rapidly (to 70.0% by 1977). With the introduction of Populus trichocarpa Torr. and Gray provenances, efforts were made to enrich the gene pool and enable new selections. The development of multiclonal varieties is envisaged from such selections for growth in pure or mixed stands (with other deciduous and coniferous species). Balsam poplars are considered to be better suited than Euramerican hybrids to this forest stand concept.

Multiclonal varieties may succeed better in forest stands with species mixtures, thinnings, and less intensive tending practices than in intensively managed plantations. In forest stands, the suppression and loss of individual trees has less effect on yield than in intensively managed plantations. In addition, balsam poplars are more tolerant than Euramerican poplars and more adapted to growth in clonal stands.

Multiclonal varieties will create problems of maintenance and control. It will be difficult to maintain a certain predetermined clonal mixture in the stock because of problems in clonal identification and differences in the rate of rooting of cuttings of various clones. In addition, it will be difficult to judge the performance of individual clones in the mixture and adjust and improve the multiclonal variety accordingly. Consequently, the concept of clonal mixtures in poplars and willows can only be conceived as a mixture of uniform monoclonal plantations of sizes dictated by site and other needs. In his study of models of clonal plantations, Libby (1980) came to a similar conclusion that monoclonal plantations are frequently the best strategy, while mixtures of 2 or 3 clones are frequently the worst and rarely, or never,

the best. Mixtures of a large number of clones in either multiclonal or monoclonal plantings are as safe as seedling plantations.

EXPERIENCES IN CLONAL PLANTATIONS

Experiences in clonal plantations have resulted from monoclonal plantations of poplars and willows, with a few clones planted on large areas, various sites, over a relatively long period of time, and in a variety of plantings. The successes and failures of these monoclonal plantations with regard to performance of clones, changes of clonal characteristics in time, and pest problems will be discussed.

Exceptional clonal performance was experienced with only a few clones. Up until the 1960's, virtually millions of ortets and clones were screened within the framework of many breeding programs and only several hundred clones were retained for propagation. Mohrdiek et al (1979) reported that in West Germany from 1948 to 1967, five hundred and fifty-six crosses were made and approximately 1.0% of the seedlings were retained for selecting promising clones. Very few of the several hundred tested clones equalled or surpassed the performance of standard clones. Thus, new clones of the quality of I-214, Robusta and Androscoggin were difficult to obtain. It is not surprising that these outstanding clones were so widely distributed.

Some of the exceptional clones adapted to a large variety of sites and growing conditions. A good example is I-214, which outperformed other clones (including local ones) in various countries of Europe, Asia, and the New World. Naturally, its ecological niche has limitations; in the north, frost limits its growth, while in southern latitudes, it is outperformed by clones adapted to shorter days. This is the situation in the southern United States where local selections of P. deltoides outperform most Euramerican poplars, including clone I-214. A change in photoperiod, as well as sites and growing conditions, limits the performance of clones. On forest sites and in forest stands, clones of balsam poplar origin, which are less demanding and more tolerant than black poplars, will outperform many clones from the latter group. In such conditions, Androscoggin is preferred to I-214. Thus, the outstanding clones performed well, but only within the range of characteristics determined by species and parent trees from which they originated.

The same clones used over a long period of time have not aged and their characteristics have not changed. P. x euramericana cl. serotina Hartig is an early selection described in 1775. More than two hundred years later, its stock is still in use, and its clonal characteristics and growth pattern appear to be unchanged. The same applies to very widely used clones, such as Robusta (selected in 1904) and I-214 (selected in 1940). This phenomenon can be explained by the well-known ability of poplars and willows to rejuvenate when grafted onto young stock or rooted from cuttings. Unfortunately, this is not the case with many other tree species, where ageing, associated with symptoms of cyclophysis, may present a problem.

Clonal pest resistance has not changed over a long period of time unless new strains of pests appeared. Poplar clones, selected in western Europe for their resistance to leaf rust (Melampsora spp.), have remained virtually immune for many years (Steenackers, 1972). However, some of the same clones became susceptible when moved to North America and exposed to different species and strains of leaf rust. Clones in cultivation in Lombardy (Italy) in the 1920's succumbed to a newly spreading disease (Venturia populina (Vuill) Fabr) causing spring defoliation; however, clones subsequently selected for resistance to the same disease remained resistance for over fifty years. Marssonina brunea (Ell. and Ev.) Magn, a fungus causing browning of leaves and green shoots first described in North America and noted in Europe in 1958, caused serious problems in monoclonal plantations in Europe in the 1960's. More than one-half of the clones and plantations suffered serious set-backs from the disease. Since then, new clonal selections resistant to the disease were found and are replacing the susceptible clones. It is obvious that pests can move from surrounding natural vegetation to monocultures, new strains of pests can develop, and new pests can be imported from other countries and continents. The problems created are easier to surpass in short-rotation than in long-rotation monocultures, especially if the former are supported by active breeding programs. However, multiclonal plantations combined with continuous phasing-out and introduction of clones can lower the risk even further and represent a more efficient method of pest management.

Monoclonal plantations of poplars and willows have thrived for more than a century. Despite periodic pest problems, they have been successful. Monoclonal plantations are easier to manage than multiclonal plantations; fewer clones cause less problems in stock production, handling and varietal control. The determination of clonal mixtures and matching of clones to sites can be a difficult and complicated task. Therefore, is it worth doing all the additional work associated with multiclonal plantations? What other benefits, in addition to better pest management, can be derived from such plantations?

The advantages of multiclonal plantations, in addition to lowering the risks of pest problems, are in utilizing significant variations in clonal performances by matching these to sites, plantation systems and production goals.

Clone-site trials in Ontario (OMNR, 1983) demonstrated that the quality of the soil of the planting sites significantly changed over a small area; systematic analysis of the planting sites was necessary to map these changes. The performance of the clones at four and five years-of-age

Clones can be chosen effectively for different plantation systems. For example, fastigiate and shade tolerant clones are better adapted to dense plantings, and clones demonstrating rapid juvenile growth are better adapted to biomass plantations. The ranking of clones in trials in Ontario changed for a period of five years. Clones which initially grew well were surpassed by other clones when spacing allowed

for such competition (Zsuffa, 1975). The errors in selection for height growth alone would have amounted to almost 50.0%.

Clonal biomass qualities vary significantly and large gains can be realized by the proper matching of clones to production goals. Clonal variations in poplar biomass qualities were demonstrated in Ontario by Anderson and Zsuffa (1975, 1976, 1982, 1983). Very significant clonal differences (sometimes more than 50.0%) were observed in many qualities of wood, bark and foliage (e.g. specific gravity, fibre qualities, gross heat of combustion, nutrient content, protein concentration and composition). The feasibility of simultaneous selection for several characteristics (such as growth rate and specific gravity, high biomass productivity and low nutrient content, etc.) was also demonstrated.

Until recently, poplar and willow breeding programs were unable to produce a sufficient number of clones of known and desired qualities for specific sites, plantation systems and production goals. Breeding programs currently underway, especially in poplar, renewed interest, and new considerations given to genetics and improvement of poplars and willows have provided hope for the realization of such goals. The genetic base for breeding has been significantly broadened, large collections of native and exotic species have been assembled and genetic variation studies are underway. As well, studies of genetic-environment interactions and parameters for many traits are yielding results. New and better planned crosses are producing rich pools of hybrids for selection and clonal development. Internationally coordinated programs, such as those within the frame of the International Energy Agency (IEA), the International Union of Forest Research Organizations (IUFRO) and the International Poplar Commission (IPC) provide good exchange of information and faster results.

A broadened genetic base, new knowledge, and good coordination can yield the desired stock for proper multiclonal management of poplars and willows. It is doubtful whether this can be achieved within the foreseeable future with other hardwood species where breeding programs do not exist or are just being initiated.

Pure clonal stands are easier for the silviculturist, the nursery man, the designated authority for control of planting stock, and the industrial user. However, if the clonal balancing is left to the user, there is the danger that one or two clones will be favoured and thus constitute the majority of plantings. In order to prevent this, each single clone should have a high and specific usability. Monoclonal plantations tailored to sites, plantations systems and production goals will be advantageous for intensive, industrial production. On the other hand, mixtures of clones, or multiclonal varieties, may be advantageous in forest stands when components, site, or their interactions are unknown (Heybrook, 1978).

CONCLUSIONS

Multiclonal plantations are a desired alternative to existing monoclonal plantations in poplars and willows. The concept of "multiclonal varieties" in individual tree mixtures may be feasible for forest stand management. The concept of mosaics of monoclonal blocks which are relatively small and similar in size in which clones are matched to soils, plantation systems and production objectives appears to be the only reasonable choice for intensively managed plantations.

Stock for multiclonal management has yet to be developed; however, active breeding projects in poplars and willows have the capacity for producing such stock. The prospects for the same development in other hardwood species are more distant.

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CONCEPTS AND EXPERIENCES IN CLONAL PLANTATIONS OF CONIFERS

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ABSTRACT

The general background of the concepts of clonal propagation and clonal plantations of conifers is discussed in the first part of the paper.

The main factors which influence production risk are reduction of genetic variation, pure plantations, little knowledge of plantations or tests, longer rotations, heterogeneity of environment, extent of plantation, exotic species, lack of knowledge about juvenile-mature correlations, and lack of basic genetic knowledge.

In the second part of the paper, results of a systematic breeding and selection program, including cutting propagation with Norway spruce, are discussed. Early testing, genotype x site interaction, and ageing with repeated repropagation are also discussed.

Clonal propagation may not only improve breeding programs, but it may also improve the transfer of the bred material to application.

RÉSUMÉ

La base théorique générale de la multiplication par clones et des plantations de clones de conifères est examinée dans la première partie de l'article.

Les principaux facteurs de risques pour la production sont la réduction de la variation génétique, les plantations pures, le peu de connaissances sur les plantations ou sur les tests, les révolutions plus longues, l'hétérogénéité de l'environnement, l'importance de la plantation, les espèces exotiques, le manque de connaissances sur les corrélations état juvénile-état mature et l'insuffisance des connaissances de base en génétique.

Dans la deuxième partie de l'article, les résultats d'un programme systématique d'amélioration génétique et de sélection, comprenant la multiplication par boutures de l'épinette de Norvège, sont examinée. On traite également des contrôles précoces, de l'interaction

génotype x emplacement, et du vieillissement accompagnant le reclonage répéte.

La multiplication par clones peut non seulement être profitable aux programmes en génétique, mais également améliorer la mise en application du matériel produit.

INTRODUCTION

Clonal plantations of coniferous species are more restricted in area than broadleafed trees. Due to the difficulties in rooting many coniferous species, most of the plantations are not very advanced in age. In Japan, only easy-to-root species, such as Cryptomeria japonica and Chamaecyparis obtusa, have been primarily planted in monoclonal plantations for a long period of time. However, vegetative propagation has progressed rapidly and has reached the point where operational clonal programs with coniferous species have been, or are being, initiated. Early clonal selection programs have been started in New Zealand with Pinus radiata (Thulin and Faulds, 1968), in California with Pinus radiata and Sequoia sempervirens (Libby and Conkle, 1966), and in Canada with spruce (Rauter, 1971). In addition, a great deal of research has been devoted to Douglas fir in Canada and the United States.

More than one million cuttings of Norway spruce have been produced annually for many years in Germany. Even more cuttings have been produced in Sweden in recent years and it is anticipated that two million cuttings will be sold next year. It appears that a re-evaluation of the concepts and experiences is necessary.

The most important literature on vegetative propagation is:

(1) IUFRO meeting on vegetative propagation in Rotorua, New Zealand, 1973. Published in New Zealand Journal of Forestry Science (4) 1974.

(2) Symposium on juvenility in Wood Perennials, College Park/Berlin 1975, Acta Horticulturae (56) 1976.

(3) Meeting on vegetative propagation of forest trees - physiology and practice, Uppsala, Sweden, 1977. Published by the Institute of Forest Improvement and the Dept. of Forest Genetics, Swedish University of Agricultural Sciences, 1977.

(4) Symposium on clonal forestry, Uppsala, Sweden, 1981. Published by the Institute of Forest Genetics, Dept. of Forest Genetics, Swedish University of Agricultural Sciences, research Notes No. 32, 1981.

(5) IUFRO meeting on in vitro propagation of forest tree species, Fontainebleau, France, 1981. Published by Associacion Foret Cellulose, Nangis, France, 1982.

(6) IUFRO meeting on breeding strategies including multiclonal varieties, Sensenstein, Federal Republic of Germany, 1982. Published by the Dept. of Forest Tree Breeding, Lower Saxony Forest Research Institute, 1982.

Within the proceedings of the last meeting mentioned above, the papers by Rauter on "Recent advances in vegetative propagation including biological and economic considerations and future potential" and by Burdon on "The roles and optimal place of vegetative propagation in tree breeding strategies" are worth reading.

In the first part of my paper, I will concentrate on the general aspects for the use of clonal plantations in breeding programs and for commercial use. In the second part, I will summarize our own experiences with clonal selection and plantations of Norway spruce.

GENERAL BACKGROUND

Vegetative propagules in conifers are generally more expensive to produce than seedling plants. Therefore, production is worthwhile only if the material is superior to seedling material, or if sufficient seedlings of similar genetic constitution cannot be produced by conventional methods.

Clonal Plantations in Breeding Programs

Vegetative propagation can be quite useful within breeding programs (e.g. for seed orchards, clonal archives, testing of material, special physiological research projects and studies of population architecture) (Burdon, 1982). However, I will only deal with plantations of clonal material which are also established for wood production.

Vegetative reproduction is only one means for propagation. However, it should never be used alone in a breeding program as this would limit progress. For long-term breeding (Kang, 1982), generative propagation must be used as the main line of a program and vegetative propagation should be used as a supplement.

In production populations, clonal propagation can be a powerful tool for utilizing, or even improving, genetic gain. Clonal selection and testing can provide considerable progress compared to bulk propagation of bred material (e.g. seed orchard progenies, controlled-pollinated families). These clones can be used at the same time for future breeding work.

The main restriction of coniferous species is the ageing process. It not only influences rootability, but also topophysis effects, growth vigour, physiological behaviour and root intensity (Roulund, 1979; Olesen, 1978). Up to now, only limited progress has been made in the field of rejuvenation of conifers (Franclet, 1981). Hedging of ortets and serial propagation are two common ways to delay ageing in conifers. Hedging, which means repeated pruning, has been successfully applied by

Libby et al. (1972) and Libby and Hood (1976) in Pinus radiata. Repeated repropagation of Norway spruce has been used in our program for fifteen years with limited or doubtful success. Another approach, used by Rauter (1982), is the propagation of very young seedlings. However, this is not clonal selection in the first stage, but mass propagation of superior and rare seedling material; a very interesting tool for practical forestry and forest tree breeding.

Concepts and Experiences for Production Populations

For most foresters, it is important to have clear and well-founded concepts for the utilization of clones in silviculture. The criteria on which their decisions are based are high production with minimal risk, and reasonable economic return.

The productivity of the clones must be guaranteed by the breeder. The extent to which he is able to do this is the basis for economic return. Risk depends on a number of factors which are influenced by the species as well as by the silvicultural concept. Production risk increases with reduction of genetic variation, pure plantations, little knowledge of plantations or tests, longer rotations, heterogeneity of environment, extent of plantations, exotic species, lack of knowledge about juvenile-mature correlations, and lack of basic genetic knowledge.

Reduction of Genetic Variation

Most clonal plantations have less genetic variation than seedling populations of natural stands. The most extreme example would be a monoclonal plantation. Many variations, from monoclonal plantations to clonal mixtures of several thousand clones, which was the method used in our early selection stages of Norway spruce, are possible and have been proposed. Libby (1981) suggested that there may be a small, optimal number of clones on the basis of theoretical assumptions. This may be true in theory, however, in practical programs, there are many arguments for keeping the number of clones high. Very limited sound information exists on this question. Hühn (1983) initiated a study from a theoretical point of view and some experimental data will be presented in this paper.

Finally, the objective of a clonal program could be to simply reproduce a seedling population. In this case, there would not be any difference between generatively and vegetatively propagated material. Seedling populations can also have a narrow genetic base, such as full-sib families. Arguments for their utilization parallel the arguments for clonal plantations of the same material.

From a risk point of view, the mixture of good clones with seedling plants of the same species, or with other species, in a systematic pattern may be advantageous. Such a system enables expensive and rare clonal material to be used without increasing production risk or reducing economic return. Such systems have been used with timber species, such as Sequoiadendron giganteum, and with Norway spruce clones selected for frost hardiness in Germany.

Knowledge of Clonal Performance

The knowledge of clonal performance of most coniferous species, especially those produced by repeated repropagation, is very limited. On the other hand, there is a large amount of seedling material available from breeding programs which cannot be reproduced sufficiently by generative means.

The differences between seedlings and cuttings have been described for those species which have been vegetatively reproduced for many years. There are species with a pronounced topophysis effect in cuttings. Araucaria heterophylla is one extreme example where cuttings from branches of second order remain branches of second order, branches of first order which are rooted remain branches of first order, and only terminal buds ofthe leader, or the leader itself, develops into a complete tree. Most Abies species show a strong topophysis influence. Cuttings of mature trees may maintain the branch habit for more than thirty years while others change to orthotropic growth after a number of years, often starting from an adventitious bud. However, severe trunk deformations can result.

Although not as common in Abies species, plagiotropism in Douglas fir is a limiting factor of a clonal propagation program. Cuttings from young ortets change to orthotropic growth within a few years and after seven years, it is difficult to see the differences between a seedling and a cutting plant. If the age of the ortet exceeds ten years, the change to orthotropic growth may take so long that permanent trunk deformation results (Vieitez et al., 1977).

The differences are less obvious in the genera Larix, Pinus and Picea, however, minor differences between seedling plants and rooted cuttings have been described for these species. Generally, the differences increase with the age of the ortets and topophysis effects partially account for the differences (Fielding, 1970; Roulund, 1979; Kleinschmit, 1978; Kleinschmit and Svolba, 1980). In Pinus radiata, the stem diameter of cuttings was lower than that of seedlings of comparable height. The diameter of cuttings of Norway spruce was greater. In a recent study, we found that Larix had a better root system in seedlings than cuttings. Norway spruce seedlings and cuttings had comparable root percentage, however, considerable clonal variation existed in root percentage. There are differences between seedlings and cuttings in root collar diameter and in a number of branches of first and second order.

These few examples may be sufficient to stress the importance of the species influence on clonal performance. Before starting an intensive clonal propagation program, sufficient knowledge of clonal performance compared to seedling plants should be obtained. As a rule, differences between seedlings and cuttings will be less with very young ortets and risk is likely to increase with ortets of advanced age.

Rotation Time

Limited knowledge of clonal performance and a limited testing period are serious problems for species grown in long rotations. For Christmas tree growing or short-rotation forestry, such as pulp wood production, a shorter testing period is acceptable whereas valuable timber production requires a rotation period of one hundred years or more. The period of testing should be realistically related to rotation time. It is difficult to establish general rules since some characteristics, such as flushing and bud set, may be judged at a very early age. If the aim of the breeding program is to improve these characteristics, early selection from locally-adapted populations are sufficient. Other characteristics, such as wood quality, snow-break resistance, or straightness of stem require a much longer period of observation and, therefore, early selection can be quite misleading. With respect to growth, it is necessary to follow the clonal performance at least until competition counterbalances the differences in vigour.

Heterogeneity of Environment

Clonal testing can only be performed on a limited range of sites. The more uniform the growing conditions, the easier it is to select clones which will reproduce their growth at other locations. For example, it is more difficult to select clones for Norway or Switzerland than for the pine region in the lowland of Northern Germany.

Genotype x site interactions are more strongly expressed on a clonal level than within families. These interactions account for approximately 50.0% of the total variation in Norway spruce clones. Genetic variation, as a buffer to environmental heterogeneity, does not exist on a clonal level. On the other hand, different clones show pronounced differences in their ecological stability (e.g. ecovalence). This may be partially due to differences in the degree of heterozygosity. This supports the concept of combined selection for vigour and stability. The risk of failure can be counterbalanced by a mixture of clones with different ecological adaptations.

Extent of Plantations

There is a difference if a selected clone is planted in a home garden or if it covers 10.0% of the woodland area of a state. Risk increases with the extent of the area of the plantation. In our clonal tests with Norway spruce, obvious clonal differences in susceptibility to diseases were found and only appeared if a clone occurred in high frequency. Pathogene populations can easily build on a homogeneous substrate. The strategies for resistance breeding, including clonal propagation, have recently been discussed by Thielges (1982). Either a strategy of slowly increasing the plantation area while increasing knowledge about a certain set of clones, or a long period of intensive testing which may cause ageing problems, is necessary if large areas are to be planted. A limitation of the maximum number of ramets per clone is one method of controlling the extent of the plantations.

Considering both risk and the logic of a breeding program, it would be wise to use flexible systems for conifers. This may include shifting clonal composition and different clone numbers depending on the progress of testing time and kno ledge.

Exotic Species

The risk of failure of clonal plantations increases with those species where little is known about their performance under plantation conditions. The history of growing exotic species in Europe provides examples of originally promising results ending in disaster, even with seedling populations. Adaptation to a certain environment is important not only in conventional breeding but even more so in clonal forestry. Recent observations in Sweden indicate that on a provenance level, adaptation to local climate only appears at an age of more than twenty years (Karlsson, 1983). We found that in Norway spruce, early selection from provenances which are not autochthonous is much less effective than from indigenous material. This is probably the case with hybrid material which can be regarded as exotic in so far as the specific combination of genomes has never been tested under the ecological conditions of the planting site. The probability of failure of hybrids increases if the ecological conditions of the parent species, especially photoperiod and length of vegetation time, deviates significantly from those of the planting site (Dormling et al., 1974).

Lack of Knowlege about Juvenile-Mature Correlations and Other Genetic Parameters

For species which have been in a breeding program for many years, basic information regarding juvenile-mature correlations and other genetic parameters is available. For these species, early selection for a clonal program is more soundly based. It must be stressed, however, that correlations calculated from populations cannot be used in clones since individuals may react differently than a population (Kleinschmit et al., 1981). On the other hand, this is a possible advantage for a clonal selection program (e.g. to select correlation breakers).

Administrative Regulation Concepts

The above considerations led to the first administrative regulations for the use of clones of coniferous species in Sweden (Hedström and Krutzsch, 1982). These regulations are well-founded and consider bothdifferent and important aspects, and are a good base for similar regulations being developed for different European countries. These rules are as follows:

(a) Clone-identified material may only be used in mixtures of clones

(b) Minimum numbers of clones and maximum numbers of ramets per clone depend on the duration of the clonal tests (Table 1)

(c) Bulk propagation of seedling material is allowed for propagation of approved forest reproductive material where the seed supply is poor

(d) It is not necessary to maintain clonal identity

(e) Bulk propagation of previously clone-identified material is permitted under certain conditions. In bulk propagated material, less than one hundred ramets per seedling are permitted. The reproductive material must contain at least twenty full sib families with different parents or fifteen half sib families mixed in equal proportions

Table 1. Swedish regulations for clone-identified material

Testing				Application	
Test Level	Duration of Test	Minimum Number of Test Sites	Minimum Number of Replications	Minimum Number of Clones/ Mixture	Maximum Number of Ramets/ Clone
1	6	2	5	120	250,000
2	9	4	7	60	750,000
3	12	6	7	30	1,500,000

The difficulty with such a system is effective control; the concept, however, seems to be well-balanced.

EXPERIENCES WITH NORWAY SPRUCE CUTTING PROPAGATION

Background

The first Norway spruce cuttings were rooted as early as 1830 by Pfifferling (1830). Clonal propagation of Norway spruce was first initated in Escherode in 1948 by my father, Richard Kleinschmit. It was also started in other places in Germany and Europe at approximately the same time. Summaries of the experiences with spruce cuttings are given by Girouard (1974) and Kleinschmit et al. (1973).

Norway spruce is the most important forest tree species in Germany and central Europe and covers more than 40.0% of the total woodland area. One problem encountered in breeding Norway spruce was that it only started to flower after twenty to twenty-five years, even in grafted seed orchards. As a result of this delay, rapid propagation is

more desirable. We expect to receive a better return from provenance selection combined with clonal selection than from seed orchard establishment.

Program

Concept

The general concept is presented in Figure 1. The left side shows the traditional line of a breeding program using provenance testing and selection, plus-tree selection, testing, and seed orchard establishment. The first clonal selection and testing can be done at the provenance level, thus improving the best provenances by early selection in the nursery. The best individuals are selected at an early age (usually at age four) from open-pollinated families, controlled-pollinated families, or species hybrids. Each individual is vegetatively reproduced and included in clonal tests.

Our hybridization work with spruce has increased considerably since 1974. To date, we have attempted more than two hundred hybrid combinations out of a possible 1260 theoretical combinations. Our aim is to include up to 10.0% hybrids in clonal mixtures. If the hybrids subsequently fail, there is no economic loss; if they succeed, they can form up to 100.0% of the final crop.

The scheme for clonal selection and testing is presented in Figure 2. It shows the close integration of a general breeding program, clonal testing, and practical application. A set of clones, selected in one year (e.g. 1974) is propagated and early-tested in the nursery. After three years, the best clones are then planted in field tests which are established at three sites in single tree plots with seven replications per clone. Ramets of these clones are already being used for practical mass propagation. For this purpose, a number of propagation units have been developed in state forest nurseries with a total capacity of approximately one million cuttings annually. The selected clones are repropagated and new clones enter from the breeding program. The information collected from the field tests is used for the reselection of the clones in the nursery three years later and the first set of clones is replaced by the next in application. The best clones from the clonal test may be subsequently used for advanced breeding material in controlled crosses.

This system guarantees the quick transfer of breeding results to application without greatly narrowing the genetic variation at an early stage. If ageing becomes a severe problem in advanced propagation cycles, the older clones can be stopped in propagation and a shift in clonal composition subsequently results. This is advantageous in maintaining genetic variation, however, it limits possible genetic gain.

Application

This system has been applied since 1968, and in 1980, we reached

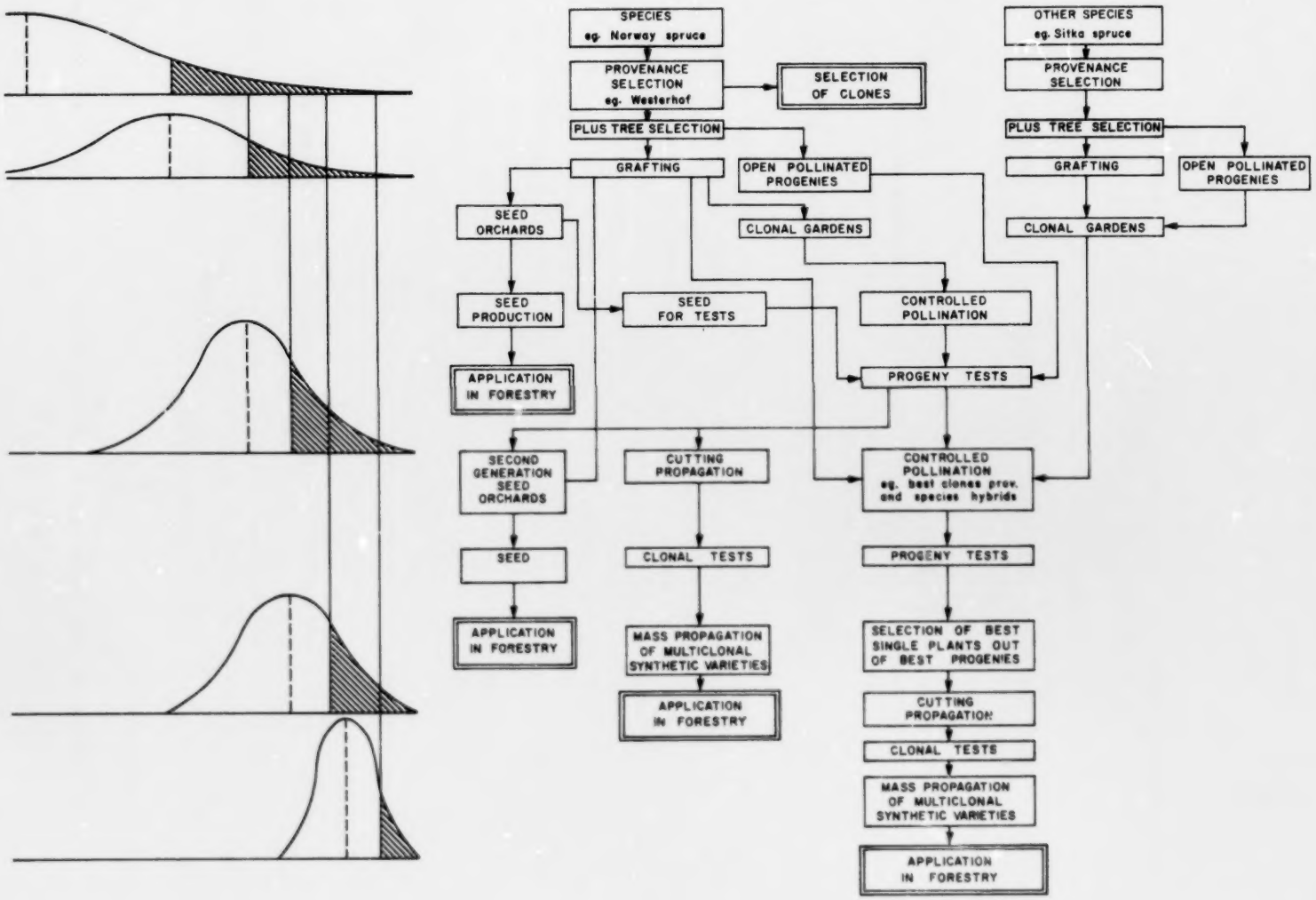

Figure 1. General concept of a breeding program

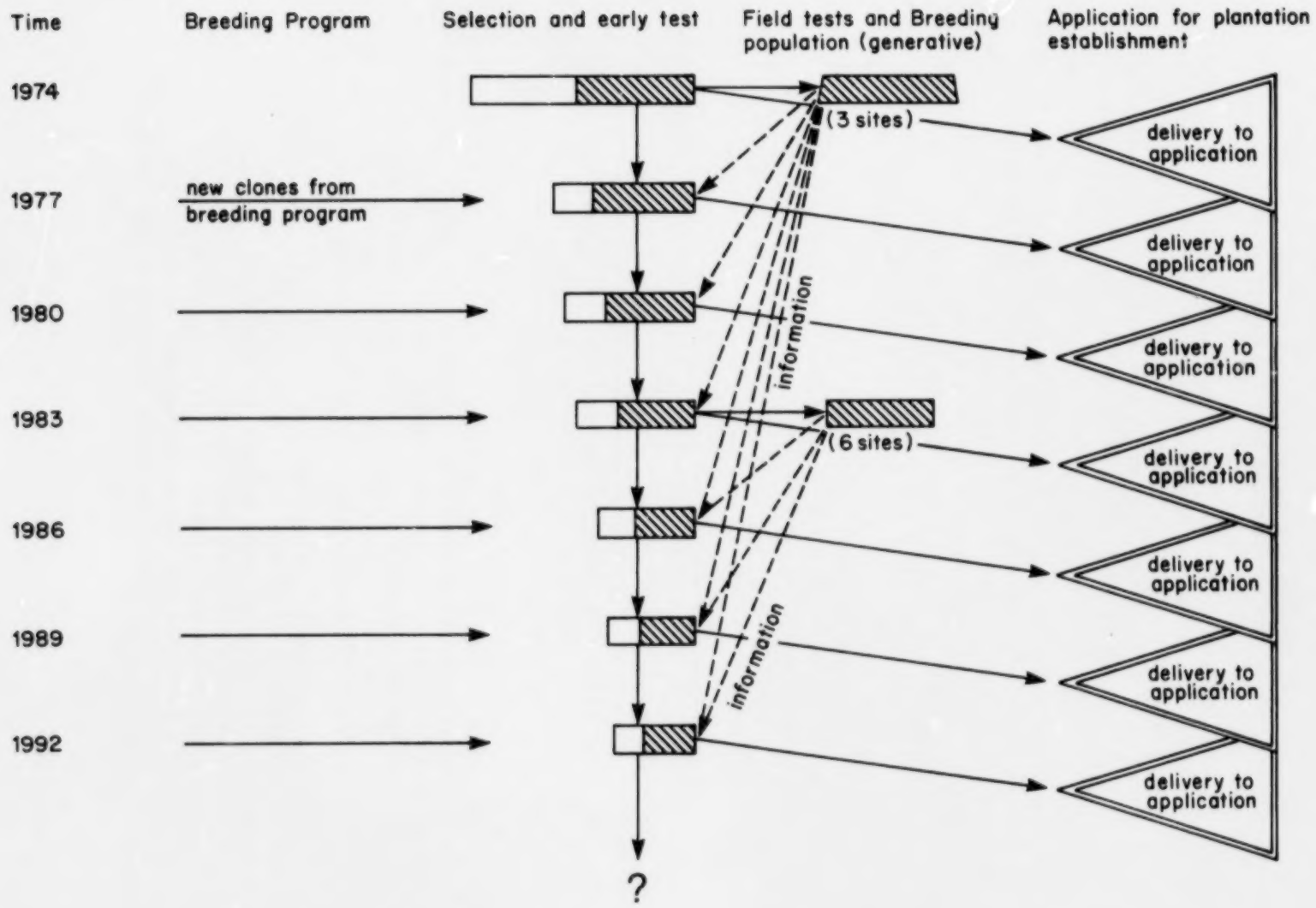

Figure 2. Scheme for clonal selection and testing

the fifth propagation cycle. To date, approximately 55,000 clones have been selected, one-third of which are still under propagation.

More than one hundred and fifty hectares of clonal tests, with single tree plots, have been established and the number of hybrids in our tests is increasing. The annual plantation area of clonal mixtures is approximately four hundred hectares. Clonal mixtures contain one thousand to five thousand clones, depending on the progress of selection. They are planted in pure stands with 2.0 x 2.0 m spacing, which is approximately ten times the number of the final crop and 20.0% less than in seedling plantations. Clonal identity is only maintained in our breeding and selection program.

The method used for application is bulk propagation of the clones which we developed. We have established seven propagation units; only three other units exist in Germany and belong to other state forest tree breeding institutes. Most of the clones originate from selections of tested provenances. However, the number of clones selected from half sib or full sib families is increasing. On the practical side, to date there have been only limited difficulties as we had good control of the propagation units and material.

The main problems of this type of program are early testing, genotype x site interaction, and ageing. We have collected a great deal of information on these problems and some of our results are presented in the following sections.

Early Selection and Testing

In 1968, we initiated an early selection program using material from Langlet's international provenance experiment. We selected thirty four-year-old clones from ten different provenances. Each provenance was represented by ten clones of inferior plants, ten clones of mean plants, and ten clones of superior plants.

We followed the growth of the ortets and two sets of ramets taken in 1970 at age six, and 1971 at age seven. The results are presented in Figures 3, 4 and 5. These results show that significant progress can be made at an early age by intensive selection, however, this is not without exception. Provenances from climatically-different regions, such as Kolonowskie, Poland, may behave quite contradictorily which can only be explained by different adaptational behaviour.

As growth continued, changes in rank took place. The amount of change was due to differences in the test and nursery environments. If these environments are similar, rank changes are comparatively small, however, they can be quite drastic. The correlations calculated for a series of field experiments are presented in Table 2 and Figure 6. Similar results are described for Norway spruce by Dietrichson and Kierulf (1982). However, as these figures are based on clonal means and use ranking, they exaggerate the real changes.

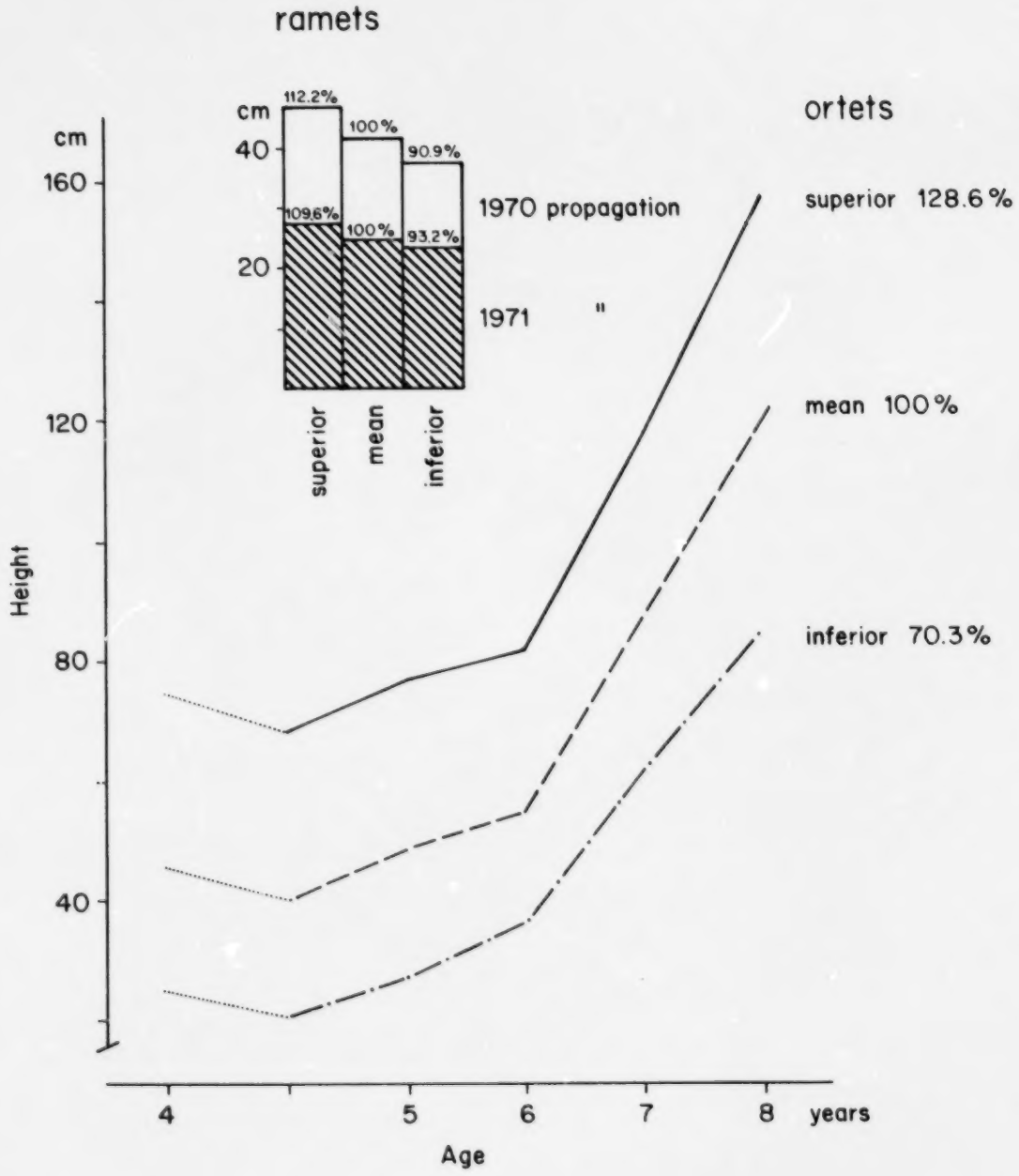

Figure 3. Early selection of Norway spruce
Langlet overall-mean
(10 provenances with 30 clones each)

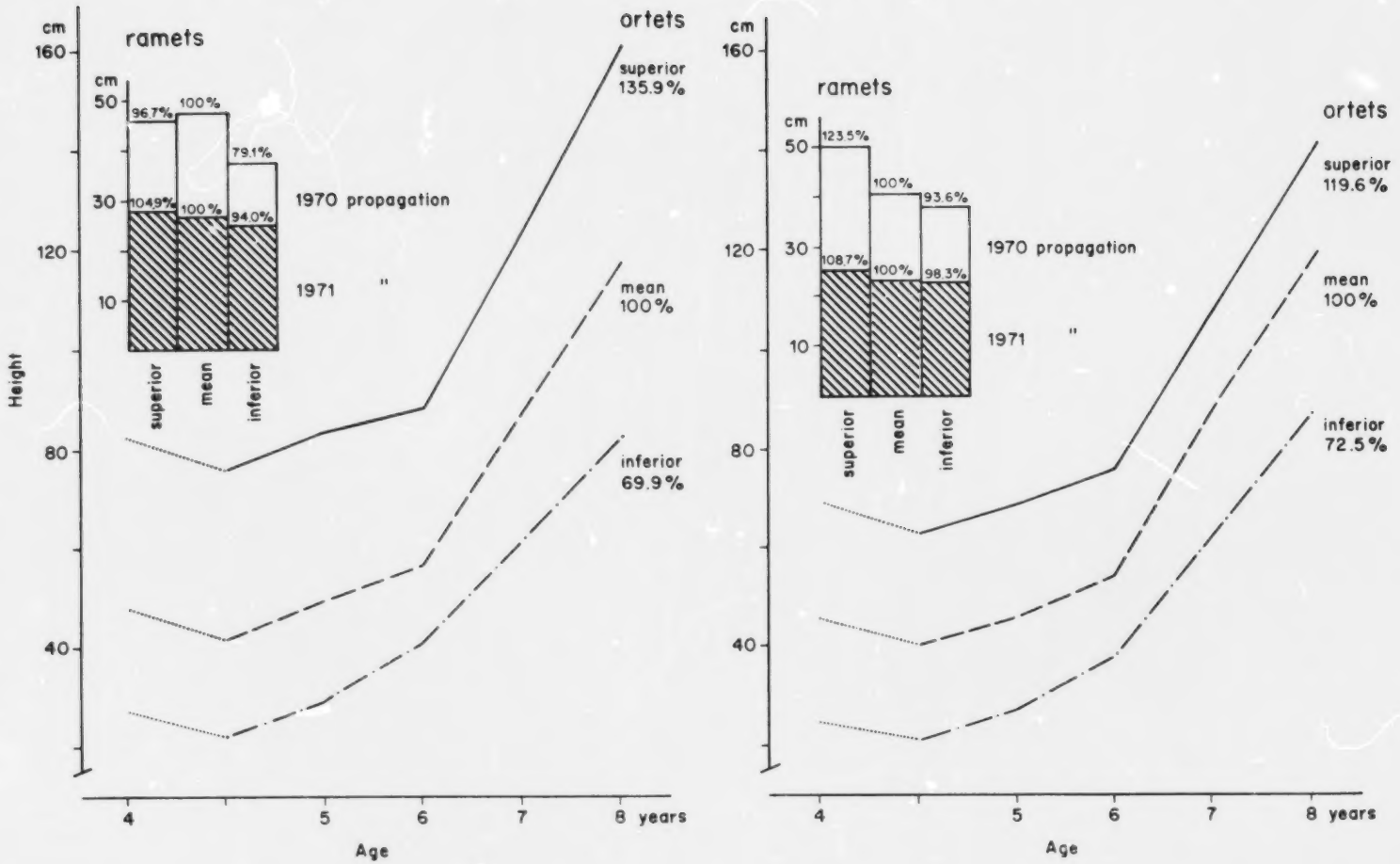

Figure 4. Early selection of Norway spruce Provenances from Poland and Czechoslovakia

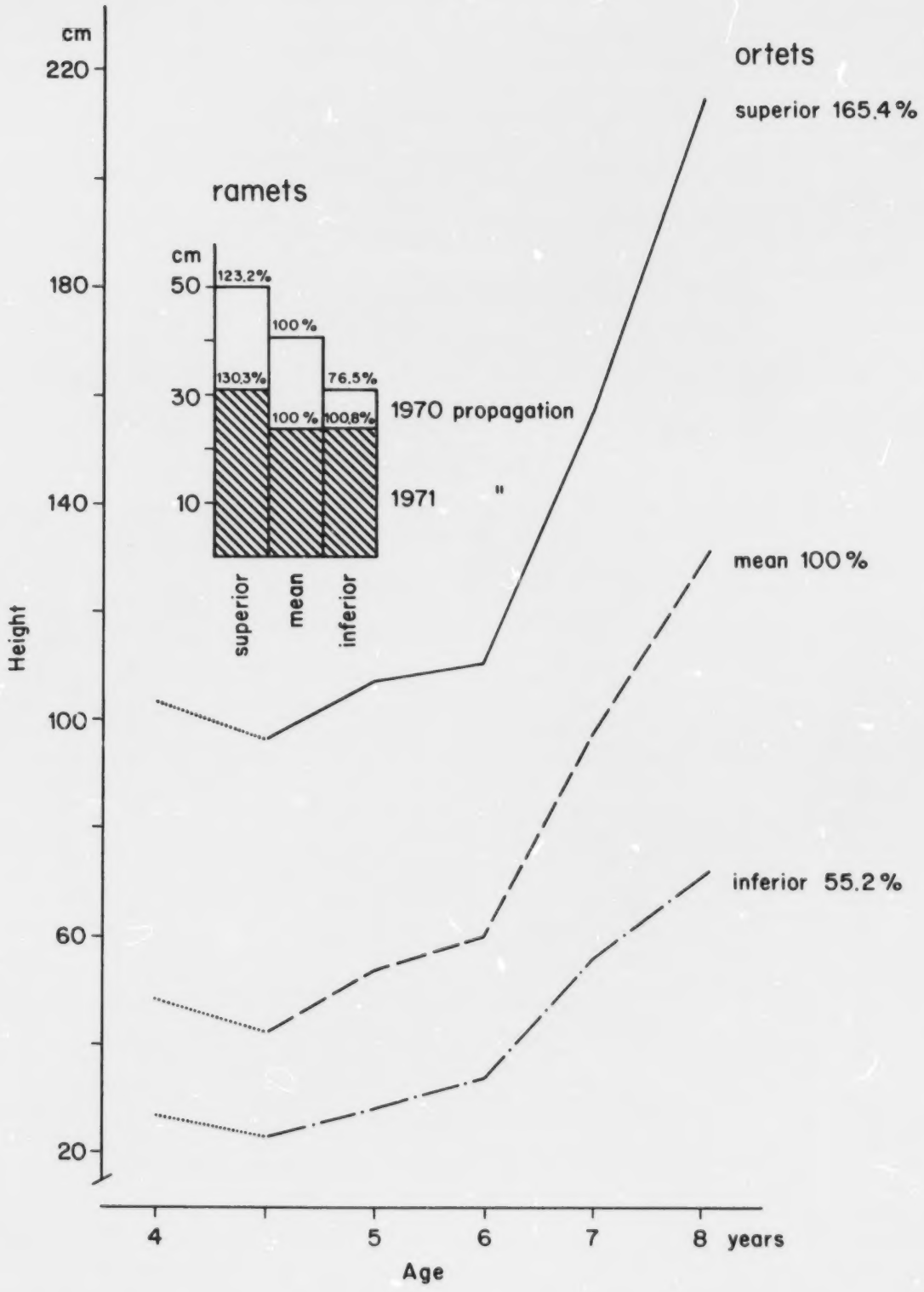

Figure 5. Early selection of Norway spruce
Provenance Westerhof

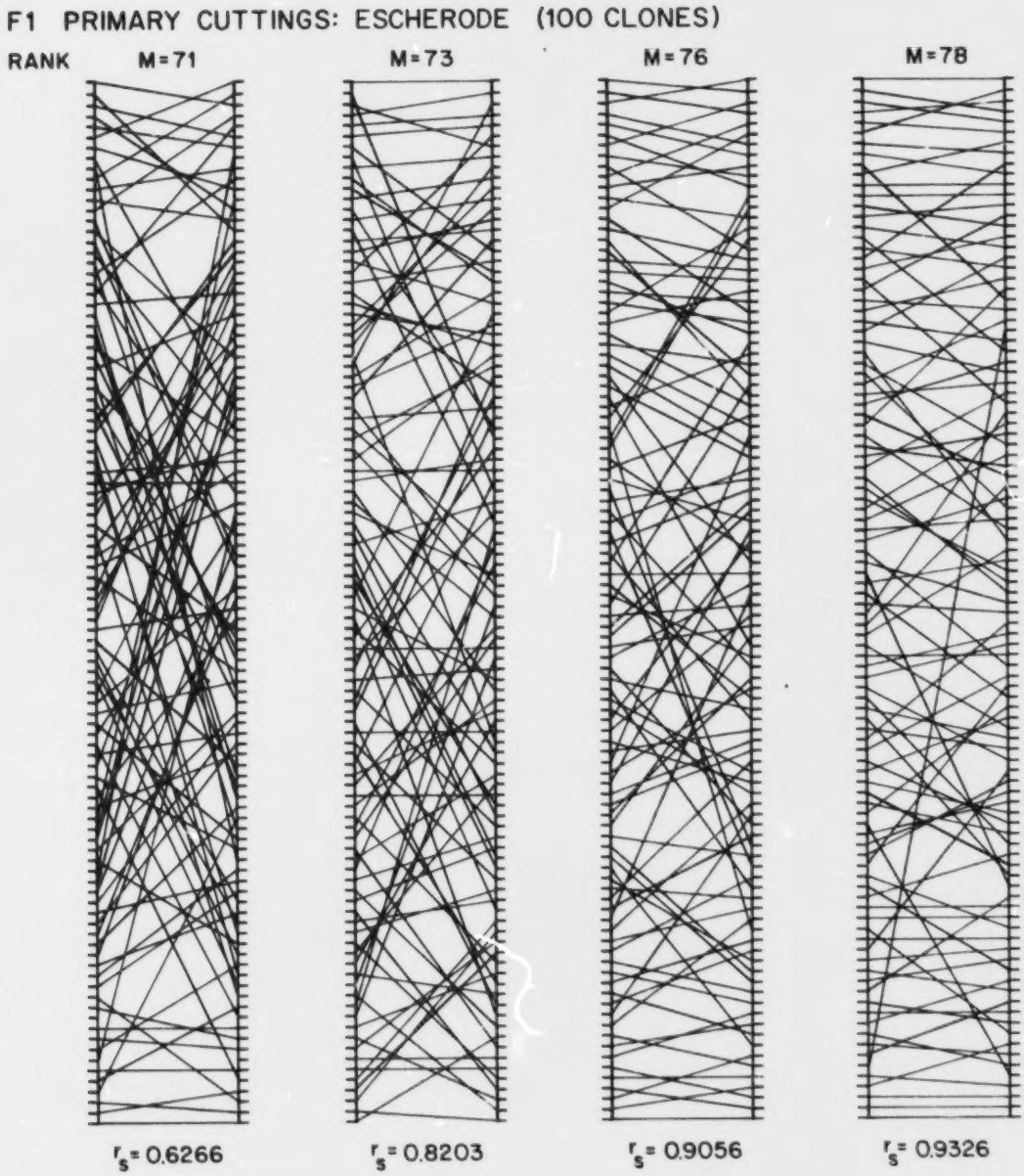

Figure 6. Norway spruce clones - Changes of ranking with age

Table 2. Correlations for clonal height development on twenty field experiments from age 3, 6, 8 (N = 61, 36, 21)

	3 - 6	6 - 8	3 - 8
	0.69	0.90	0.52
	0.82	0.80	0.57
secondary	0.82	0.80	0.69
	0.52	0.84	0.30
	0.70	0.84	0.44
	0.84	0.50	0.20
	0.62	0.85	0.55
	0.76	0.70	0.36
	0.65	0.64	0.28
tertiary	0.83	0.48	0.13
	0.84	0.53	0.16
	0.81	0.56	0.23
	0.63	0.44	0.30
	0.81	0.70	0.35
	0.84	0.70	0.54
	0.69		
	0.86		
	0.85		
	0.76		
quatenary	0.80		
	0.84		
	0.81		
	0.81		

Details of rank changes are not very clear. They may fluctuate or go in one direction. Some clones maintain their positions while others change drastically. This may be due to changes of annual weather conditions (frost damage, drought, etc.), developmental differences, or genotype x site interactions.

Early, final decisions cannot be made due to rank changes which occur in the first years in the field. Rank changes between clones depend primarily on the age of the plantation. They are quite drastic in the first years after establishment and more or less disappear after ten to twelve years-of-age after which, changes only occur in one direction, from up to down, due to competition in the plantation.

We were interested to see how correlations change if groups of clones as in a clonal mixture were considered. We formed groups of different sizes beginning with one clone and increasing the number of clones stepwise. As expected, the genetic variation slowly increased within the groups. However, the expected gain between groups as well as the genotype x site interactions decreased. Ranking was done according to

the first measurement of the field tests and all subsequent grouping followed this early ranking. For this purpose, we evaluated our older Norway spruce clonal tests. The general results (Figure 7) show that even if one begins with very poor correlations, such as 0.3 with one clone, these improve rapidly if the groups increase; with ten clones, correlations reached 0.8; with twenty clones, 0.9; with fifty clones, 0.95; and with sixty-five to one hundred clones, 0.99 to 1.0, depending on the site. At the same time, differences in height between the groups mean decreased and gain decreased. However, gain can only be realized at an early stage if correlations are good. By increasing the group size from one to five clones, the difference between groups decrease from more than 100.0% to 15.0to 20.0%. A 15.0% gain can be achieved at the end of the nursery stage whereas a 100.0% gain requires an additional nine years. This implies all the problems connected with ageing. An additional selection of 50.0% of the best clones in the nursery would have produced an 8.0% gain; 20.0% of the best clones would have produced a 15.0% gain; and 10.0% of the best clones, an 18.0% gain. Considering the cost of field testing, this information can aid in considerably improving the selection procedure. A selection program which takes these results into consideration is presented in Figure 8.

Genotype x Site Interactions

Significant rank changes can be observed between clonal means on different sites. Genotype x site interaction is significant in all series and accounts for 8.0 to 50.0% of total variation.

The ecological stability of the clones can be quite different. We calculated different stability parameters (Wricke, 1964; Hühn, 1979) and found differences of 1:10 to 1:20 for the clones. Therefore, extreme differences are expected in adaptational behaviour of different clones (Figure 9).

All this seems quite pessimistic for the use of clones in a breeding program. The practical success, however, can be measured in the superiority of the clones as compared to seedling standards. We have as a standard in our experiments, a tested stand, provenance Westerhof, which is our best indigenous source.

As a mean figure from all primary tests, the seedling standard ranks between 75.0 to 95.0% depending on the age. The top clones reach 50.0 to 120.0% superiority as compared to the overall clonal mean. In all secondary propagated material, we reached a mean figure of the seedling standard of 85.9% and 84.9% respectively, with the best clones ranging from 31.0% to 66.0% superiority as compared to the overall clonal mean. As a mean figure of the tertiary propagated material, the seedling standard had 84.0% with the best clones showing 40% superiority as compared to the overall mean.

The quatenary cuttings only reached six years of age at the last measurement. As a result, comparisons are not very valid at this time as this material had a poor start in comparison to the seedlings which

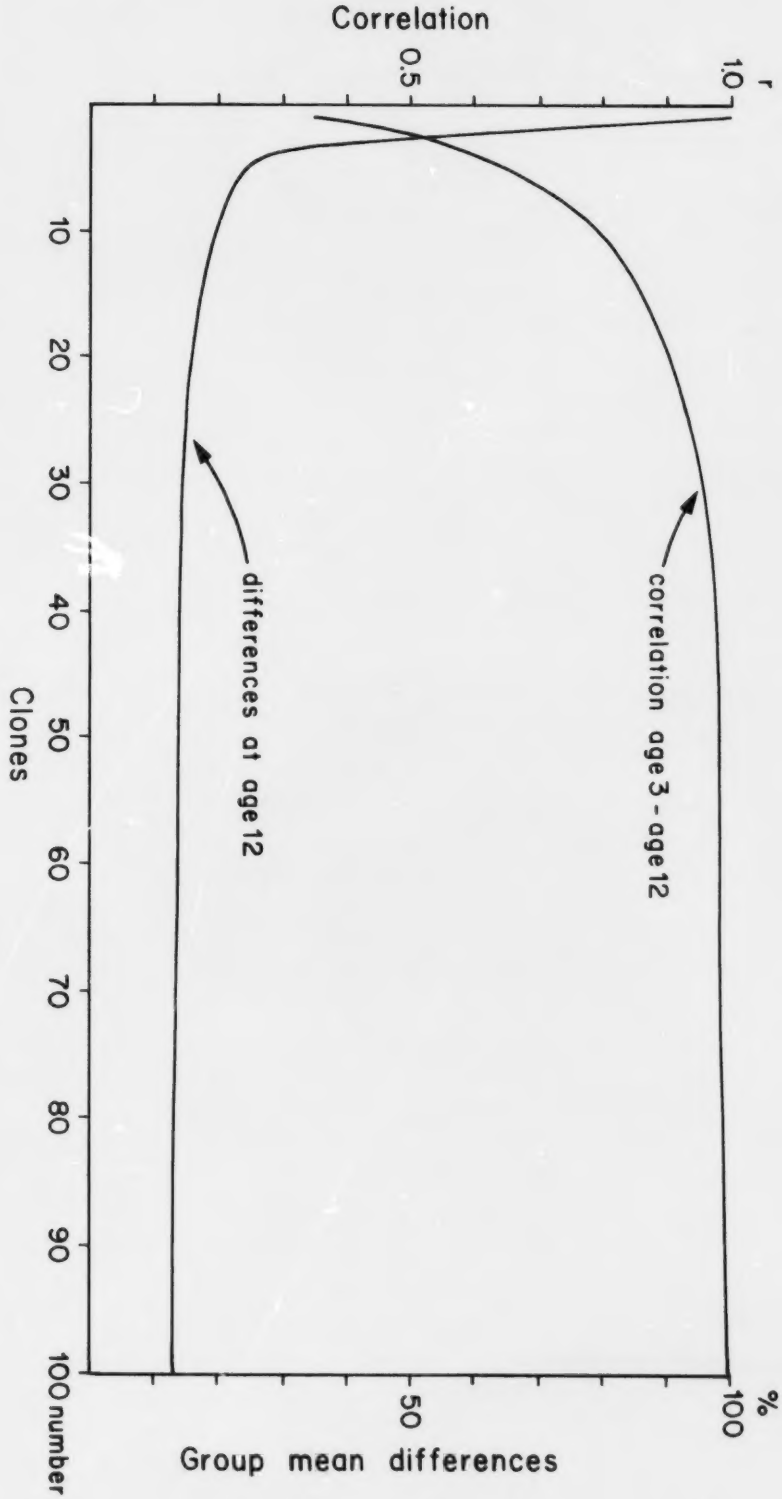

Figure 7. Changes of correlations and differences between group means for height with grop size for Norway spruce clones

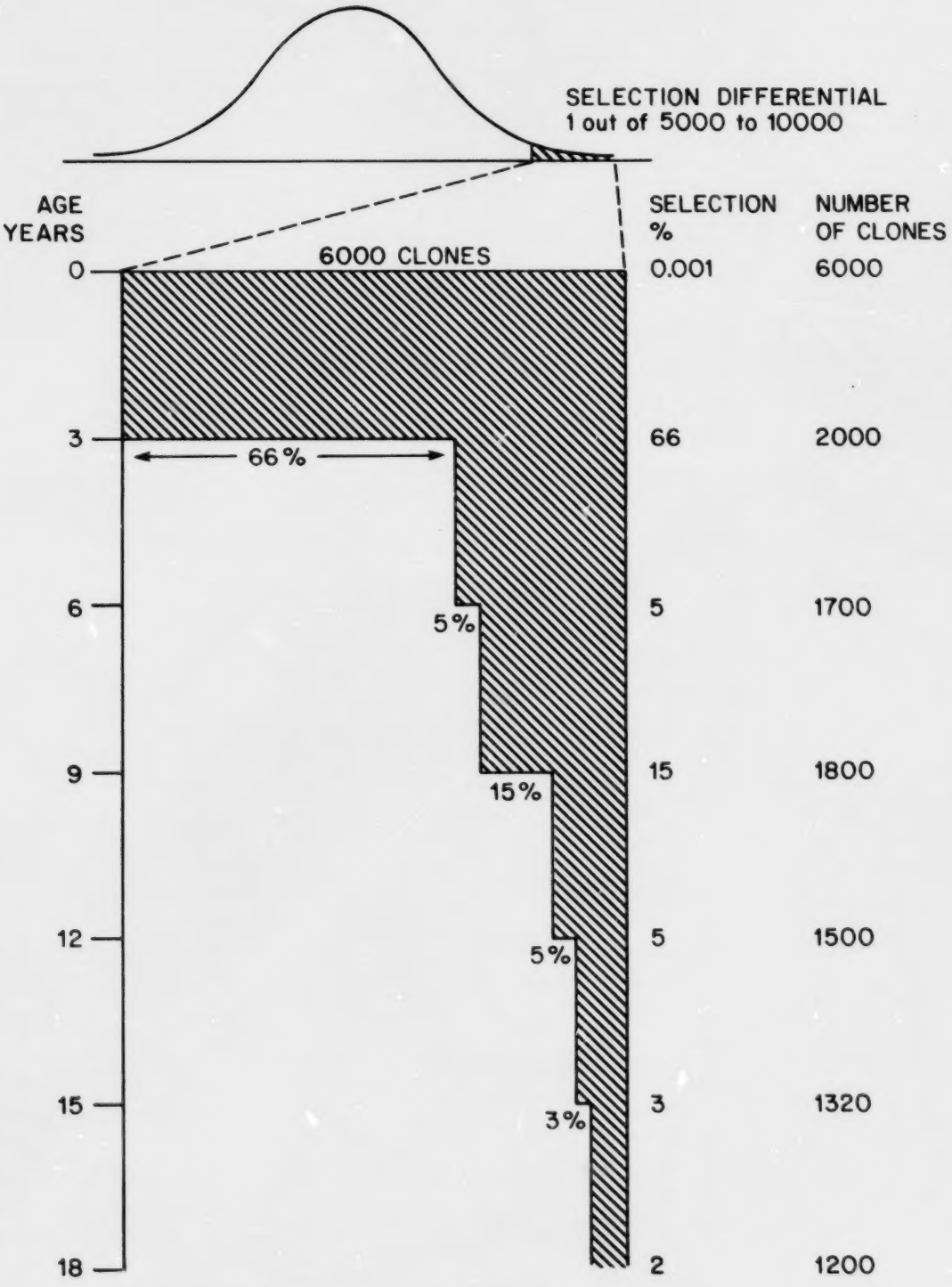

Figure 8. Proposed selection program for Norway spruce clones
3 years propagation cycle

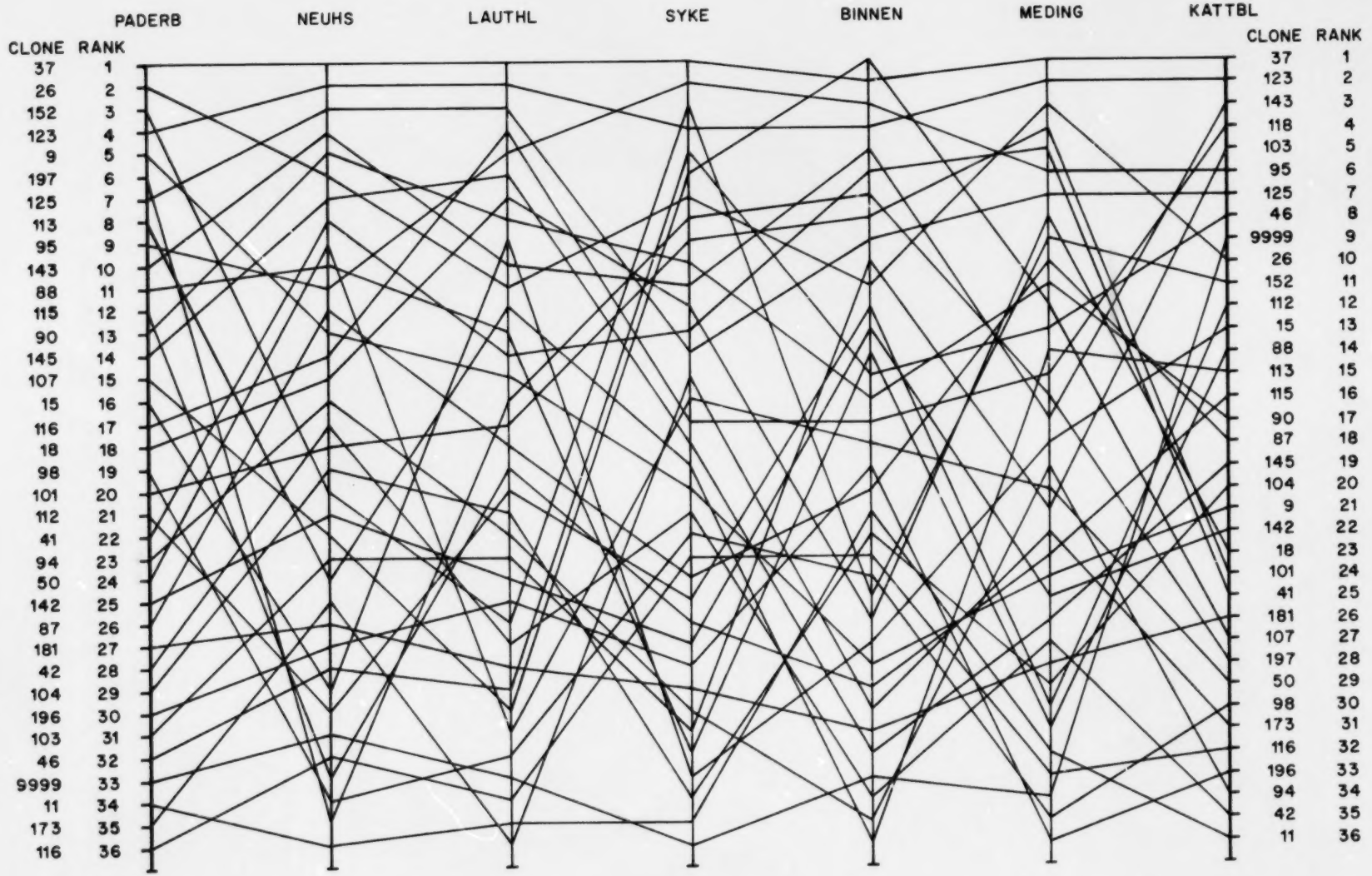

Figure 9. Norway spruce cuttings - tertiary propagation
7 field tests
Rank changes of clonal means due to location

originated from a different nursery bed and had an initial superiority which subsequently decreased.

Selection of single clones from one location appears to be quite ineffective. Therefore, we increased the groups of clones using the same method we used for different age measurements.

The following illustrates the improvment of the correlations for a set of 425 clones tested on two very contrasting sites (age 8).

Number of clones	Correlation coefficient	Group differences (%)
1	0.39	250.0
10	0.84	41.0
50	0.93	33.0
75	1.00	19.0

At the same time, differences between groups decreased. Conclusions can be drawn from rank changes due to age influences and genotype x site interactions. The gain became more stable with mixtures of clones than with single clones, however, the amount of gain decreased.

Ageing

Ageing is the most important problem in clonal programs with conifers and can have severe implications.

In the autumn of 1979, we had a large amount of material of primary to quatenary cuttings which had been aged in the nursery for three years. The different stages were represented as follows: primary, 2000 clones; secondary, 2197 clones; tertiary, 8417 clones; and quartenary, 5387 clones.

Figure 10 presents the rooting percentage and height growth in the nursery. There is an obvious increase in rooting potential from secondary to quatenary propagation. There is also an obvious superiority in heightin the first propagation cycle which may result from having a good selection base for primary material. The difference between height growth from the second to fourth propagation cycle was not significant

We took a representative sample of twenty clones from each propagation cycle, which had been replicated four times, for further analysis. The results are presented in Figures 11 and 12. There is no obvious disadvantage between the fourth propagation cycle and the second or third. The general shape, tropismus and habitus are even better. The only difference is a shift from branches of first order to branches of second order.

In 1982, we had collected material from the first to the fifth propagation cycles in our nursery. The results of height measurement are presented in Figure 13. This material is, more or less, a random sample of different propagations, only biased by the different, original basis for selection. The fifth selection, however, includes all remaining

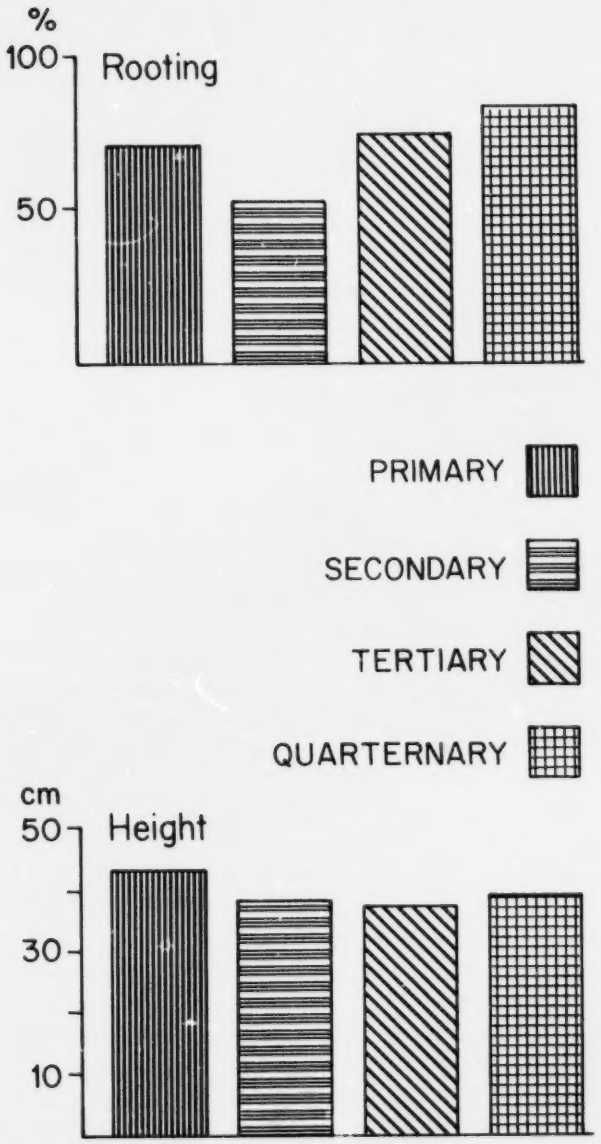

Figure 10. Rooting and height growth of Norway spruce cuttings in different propagation cycles

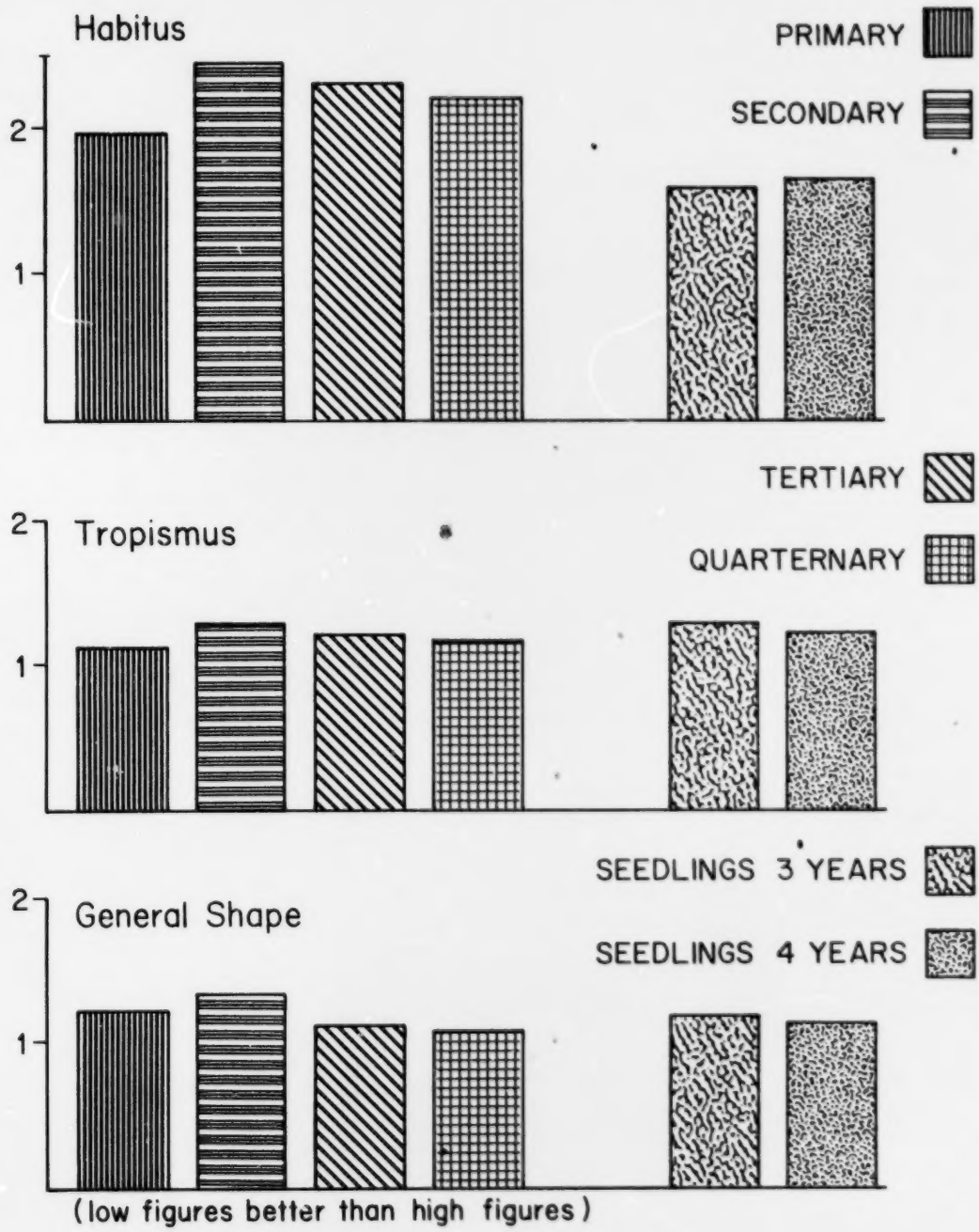

Figure 11. Structure of cuttings (3 years) from different propagation cycles and from seedlings

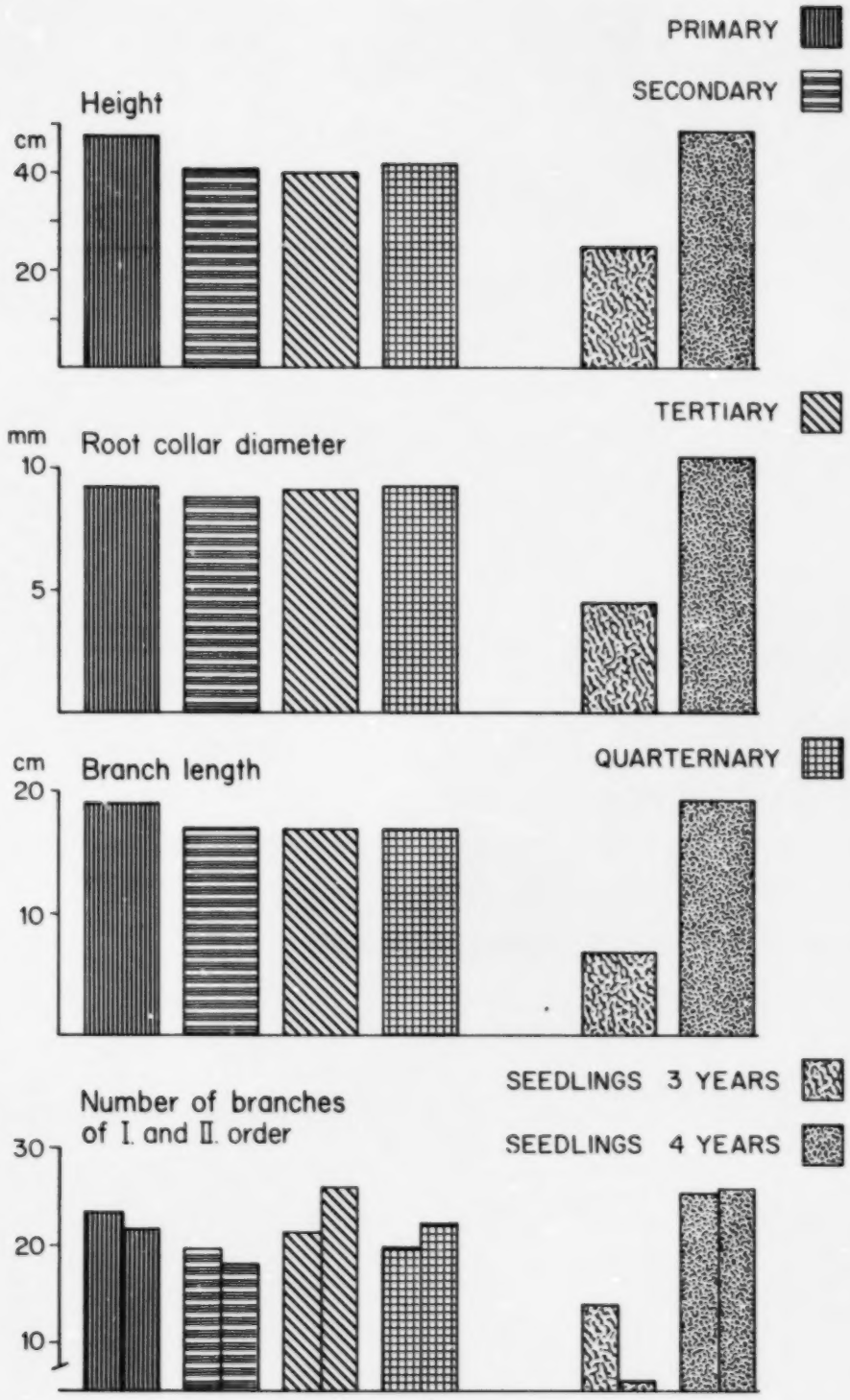

Figure 12. Structure of cuttings (3 years) from different propagation cycles and from seedlings

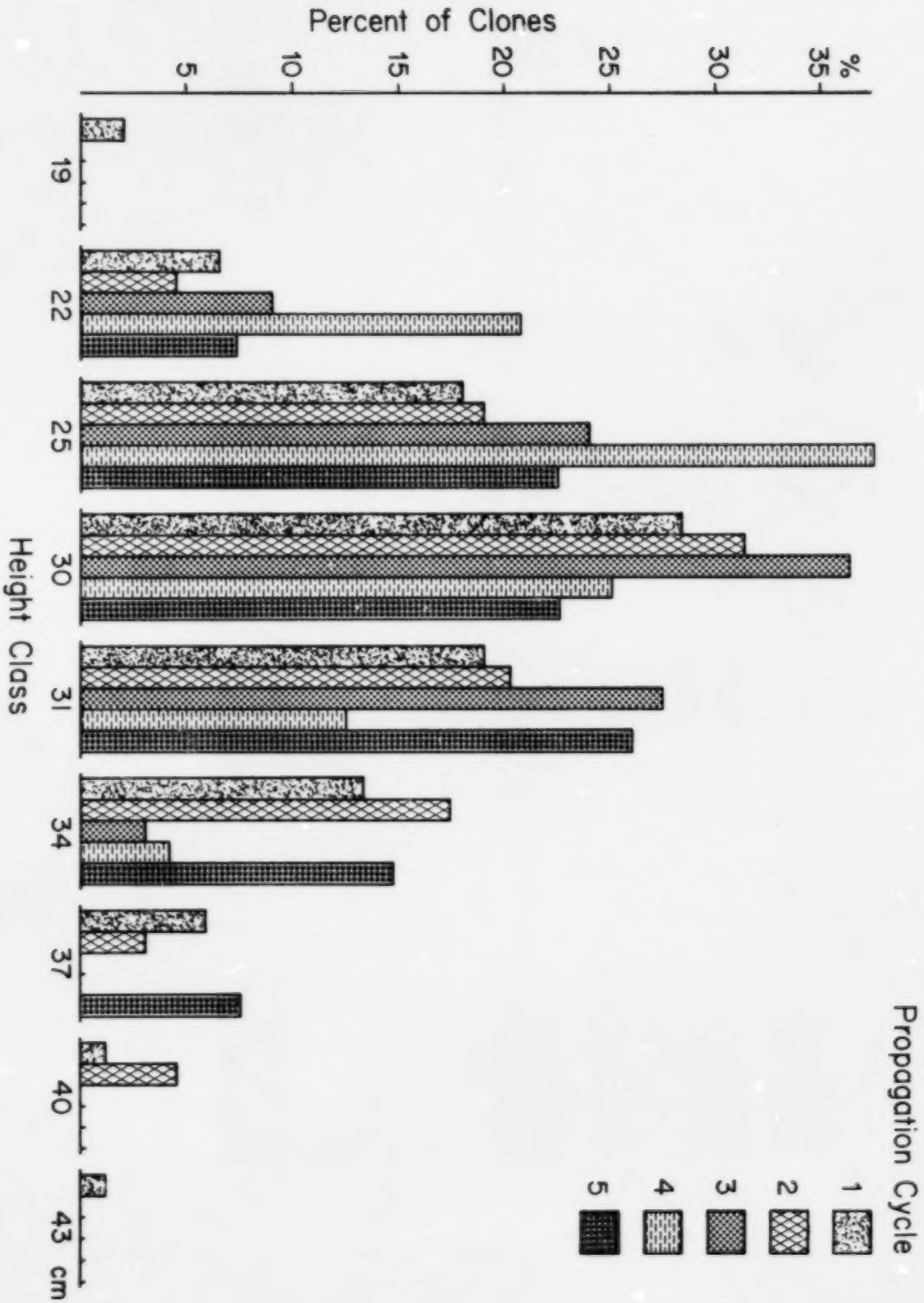

Figure 13. Height of clones of different propagation cycles (ramdom samples)

clones. The respective mean values from three replications are: first propagation, 31.1 cm; second propagation 30.4 cm; third propagation, 28.3 cm; fourth propagation 26.8 cm; and fifth propagation 30.0 cm. There appears to be a slow decrease from the first to the fourth propagation cycle. The fifth, however, is better in height than the third and fourth. The effect of higher selection intensity in this material cannot be excluded.

The above is an evaluation of developent in a young stage. Physiological activity and re-establishment of inherent growth vigour may take longer in higher propagation cycles. These small differences, however, may not be too important from the point of view of production.

Finally, we correlated the growth of the second, third and fourth propagation cycle of the same clones tested on six (secondary), seven(tertiary) and seven (quatenary) sites, with seven replications on each site. There are still significant correlations between the second and third propagation cycles for the same clones, however, there are almost none between the third and fourth.

The same is true at a more advanced age. The analysis of variance calculated with clones, propagation cycles and replications within propagation cycles generally provided low values for clones, accounting for 10.0 to 20.0% of the total variation. Propagation cycles account for 30.0 to 80.0% of total variation, and interaction clone x propagation cycle accounts for 13.0 to 64.0% of total variation, depending on the respective set of experiments and propagation cycles.

The lowest repeatability of the clones was generally found in the third and fourth propagation cycle with correlation coefficients decreasing as far as zero or -0.017. As the quatenary material experienced frost heaving in the nursery, these figures are not very important, and further development of this and other experiments including quartenary propagation should be anticipated.

If groups of clones are increased as was done for different ages and plantations sites, correlations improve with groups of five clones to 0.5, and to 0.9 with groups of ten clones. Since the total number of clones which passed the fourth propagation cycle was limited, it was not possible to experimentally evaluate at which clone number the correlations reached 0.99 to 1.0. However, group differences are much smaller with different propagation cycles than with height and location, and they did not surpass 20.0% of the overall mean. This evaluation, however, has one more weak point which must be mentioned to avoid a pessimistic view of repeated repropagation: all clones that had reached the fourth propagation cycle has passed four selection cycles too. Therefore, total variation had been reduced considerably and the comparison was made between the top clones of the species. The selection intensity at this stage was 1 out of 40,000 to 1 out of 80,000 original seedling plants (see Figure 8).

This means that rank changes are quite possibly due to small differences between clones, or low correlations could be an indication for

successful selection as well as for differences in the ageing progress of the clones. There are indications for both versions.

More information must be collected before the question of ageing of clones in repeated repropagation cycles can finally be answered.

CONCLUSIONS

Concepts of clonal selection programs in conifers are generally more difficult than in broadleaved species due to the ageing process. They require precautions that guarantee a genetic gain even if ageing is taking place.

The easiest and most secure concept is that of bulk propagation of tested superior material. However, this limits the possible gain and is not a clonal selection and testing program.

The next possibility, which improved bulk propagation material to a certain extent, is a subsequent screening at the end of the nursery phase which guarantees an additional gain anywhere between 5.0 to 25.0% depending on selection intensity. Again, this is possible without a clonal selection and testing program. An intensive clonal selection from good provenance or tested material with subsequent testing of the clones can provide considerable gain as long as the genetic base is not drastically reduced and if ageing does not counterbalance the gain. Due to errors as a result of early selection, genotype x site interactions, and genotype x propagation cycle interactions, the success of selection depends not only on selection intensity, but also on the size of the selected population of clones. It is not necessary to keep the clones separate from the applied part of the production. However, they must be treated separately in the selection and testing part of the program. A selection program should be kept open to introduce new clones if ageing becomes a severe problem. The situation may be quite different from one species to another.

Even if superior clones are detected so late that optimal propagation is impossible, they are not lost from the breeding program. They can be used in the advanced breeding population and rejuvenated by generative means. This possibility has been neglected up to now in many programs.

Production of outstanding hybrid families, and bulk propagation of these families or the best single plants selected out of these is another option. To prevent risks, these may be planted with conventional seedling material (e.g. in a percentage which corresponds to the trees of the final crop).

Finally, since tissue culture is most probably a tool for influencing a clone without disturbing the correlating systems in the plant, it may hopefully be a possibility not only for mass propagation, but also for future rejuvenation with conifers.

There is reason for optimism with clonal programs in conifers, and clonal propagation, selection, and testing are all good methods of improving the genetic gain and flexibility of tree breeding in conifers.

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SESSION II

MODERATOR:

DR. BRUCE P. DANCIK

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CURRENT STATUS OF MACROPROPAGATION

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ABSTRACT

Rooting of cuttings from forest trees, particularly conifers, has reached a level of success in recent years that encourages further development towards fully operational clonal programs. Optimal conditions for rooting involve the physical conditions of both the plant material and the rooting environment. Maturation state, type of cutting, crown position, physiological condition of the cutting, and season of collection all affect the rootability and speed of rooting. Though conditions may vary with species, a general prescription would be to use stem cuttings from the lower crown, collected in early spring from young trees in a good nutritional state. Rooting media, air and soil temperature, humidity, light intensity and chemical treatments are all external factors that may affect the rooting of cuttings. Although the technology to root cuttings has progressed rapidly, there remains a number of biological constraints impeding the rapid implementation of clonal forestry. Control of maturation state and methods of early testing are crucial problems to be solved. Questions of genetic diversity, deployment strategies and growth habit of cuttings must also be further investigated. In light of recent advances in both practice and theory of the above issues, the clonal option is rapidly assuming a larger role in operational forestry.

RÉSUMÉ

Ces dernières années, on est parvenu à induire le développement des racines des boutures d'arbres forestiers, notamment de conifères, au point de croire à la possiblité de programmes de clonage tout à fait opérationnels. Parmi les conditions optimales du développement des racines, il y a les conditions physiques dans lesquelles baignent le matériel biologique et le milieu d'enracinement. La maturation, le type de bouture, son origine dans la cime, l'état physiologique de la bouture et la saison du prélèvement influent tous sur les possibilités et la vitesse d'enracinement. Même si les conditions peuvent varier selon l'espèce, la règle générale serait de prélever les boutures de la tige, au début du printemps, dans la partie inférieure de la cime de jeunes arbres en bon équilibre nutritif. Le milieu d'enracinement, la température de l'air et du sol, l'humidité, l'intensité de l'éclairage et les traitements chimiques sont tous des facteurs externes qui peuvent influer sur le développement des racines. Même si la technique favorisant le

développement des racines des boutures a rapidement évolué, il subsiste un certain nombre de contraintes biologiques qui empêchent l'utilisation rapide des clones en foresterie. La maîtrise de la maturation et les méthodes permettant des tests précoces sont capitales. Il fait aussi examiner davantage les questions de diversité génétique, de stratégies de mise en place et de port des boutures. Compte tenu des progrès récents dans la pratique et la théorie des questions susmentionnées, les clones prennent rapidement de plus en plus d'importance en foresterie opérationnelle.

INTRODUCTION

Rooting of cuttings for the majority of tree species (particularly conifers) has been deemed sufficiently successfuly only within the last ten to fifteen years, and is only now being seriously considered in artifical regeneration and breeding programs. Some genera, such as willow (Salix L.), poplar (Populus L.), and cryptomeria (Cryptomeria japonica (L.F.) Don) have been routinely rooted for many years (Chemlar, 1974; Toda, 1974; Zsuffa, 1976). The successes of programs using these genera have provided much of the encouragement and impetus to solve the problems of difficult-to-root species and genera.

The following sections will (i) summarize the optimal conditions for successful rooting, with examples showing the variability in tolerance amongst species, (ii) outline some biological concerns and (iii) suggest areas where developmental work is needed to make rooted cuttings more economically competitive with seedlings.

OPTIMAL CONDITIONS FOR SUCCESSFUL ROOTING

Rooting success is dependent upon optimizing many endogenous and exogenous factors. The endogenous variables include maturation state of the cutting donor, type of cutting, physiological condition of the cutting, preconditioning of the cutting or cutting donor, and the season of collection. The exogenous variables include rooting medium, ambient temperature and humidity, photoperiod and light intensity, and hormone and chemical treatments.

The degree of control required for each of these variables is directly dependent upon whether the individual species is an easy or difficult rooter. Many hard pines require specific preconditioning treatments (Hare, 1978) or well-controlled environmental conditions (Hare, 1978) to obtain any degree of rooting success, while many poplar and willow species root easily, and even unrooted cuttings can be planted directly onto a plantation site (Zsuffa et al., 1977); Densmore and Zasada, 1978).

Endogenous Factors

Maturation State of the Cutting Donor

The less mature (i.e. the more juvenile) the cutting donor, the greater the success in rooting. As the state of maturation increases, percent rooting decreases. For example, juvenile cuttings of black spruce (Picea mariana (Mill.) B.S.P.) taken from twelve-week-old seedlings rooted more than 99.0% (Armson et al., 1980), whereas cuttings from four-year-old seedlings of the same species averaged 62.0% rooting with clonal variation in rooting ability ranging from 0% to 95.0% (Rauter, 1971). As the donor plant matures, not only does rooting decrease, but the length of time to develop roots increases (Isikawa, 1968) and the quality of the root system changes (Libby and Hood, 1976). Similarly, the subsequent rate of height growth decreases (Kleinschmit and Schmidt, 1977; Rauter, unpubl. data) and the effects of topophysis are more pronounced and take longer to overcome (Kleinschmit, 1961).

In Ontario, we experienced quick (in less than 5 weeks) and very efficient (better than 99.0%) rooting of very juvenile cuttings. This changed our approach in starting from more promising, mature trees-ortets and lower rooting percentages to propagating young seedlings from selected full-sib families with fast, reliable rooting. The poorer performing clones can be dropped from this program if the juvenile state can be maintained. The remaining clones can be propagated on a large scale. The quality of the resultant clonal plantations will be substantially improved over those produced from seed orchard seed.

There are several promising methods for retaining juvenility including hedging (Libby and Hood, 1976; Hartney, 1980; van den Driessche, 1983) and serial propagation (Kleinschmit, 1977; Morgan et al., 1980). However, it appears that these methods are successful with only some species. For example, serial propagation rooting percentages over a three year period decreasedless when cuttings were continously taken from black spruce donors, and more when taken from white spruce (P. glauca (Moench) Voss) donors (Phillion, unpubl. data). Also, black spruce exhibited less topophysis than white spruce.

Type of Cutting

Many recent studies confirm the results of earlier work on selecting the best type of cutting. For some species, root cuttings and brachyblast cuttings (made of shoots originating from fascicular buds) are the more successful types. Since most aspen species are difficult to root from stem cuttings, reasonable success can be achieved by taking root cuttings from the parent donor, producing suckers, and then rooting the resultant suckers (Zufa, 1971; Schier and Campbell, 1976). This is similar to the work done by Muhle Larsen in the 1940's (in Nienstaedt et al., 1958). Brachyblast cuttings from nine to twelve-year-old trees of Pinus densiflora Sieb. and Zucc., P. thunbergiana Franco, and P. rigida Mill. root significantly better than stem cuttings taken from the same trees and treated in the same manner (Hong, 1974). Brachyblast cuttings

have also been successfully used in Ontario for jack pine (P. banksiana Lamb.) and scots pine (P. sylvestris L.) (Phillion, pers. comm.).

Stem cuttings are the most common form of propagules and usually perform the best. The length of the stem cutting can affect the success of rooting. In jack pine, cuttings less than 2.5 cm in length did not root as well as those 2.5 to 7.5 cm (Zsuffa, 1974). The growth of the roots was directly proportional to the length of the basswood (Tilia americana L.) cuttings (Morsink and Smith, 1974).

The Influence of Crown Position

The crown position from which the cutting is obtained is important and becomes more crucial as the maturation state of the donor increases. Spruce cuttings taken from the lowermost portion of the crown root better than those from the uppermost portion, and laterals root better than terminals (Girouard, 1971). Roulund (1979) found that Norway spruce (Picea abies (L.) Karst.) cuttings from the lowermost portion of the crown exhibited less plagiotropism than those from the top. In redwood (Sequoia sempervirens (D. Don) Endl.), cuttings from the lower crown rooted better than those from the upper crown and the duration and severity of plagiotropism was greater in the lower crown than the upper crown (Libby, pers. comm.). Most of the evidence is consistent with the hypothesis that upper crown cuttings are in a more advanced state of maturation than lower crown cuttings.

Physiological Condition of the Cutting

Several factors determine the physiological state of the cuttings; unfortunately, only a few of these are understood. Although it is known that sufficient carbohydrate reserves in combination with a high C/N ratio favour rooting (Haissig, 1974b), the relationship between carbohydrate metabolism, root primordium development, and enzyme activity is not understood. Haissig (1982) attempted to identify enzymes and enzyme systems, and found that these were positively related to primordium development and to the metabolism of reducing sugars. He assumed that these enzyme activities had a physiological impact and influenced the speed and amount of primordium development in the cuttings.

Cuttings must have sufficient nutrient reserves to survive the rooting period until proper growth occurs (Land Jr., 1977). Without sufficient reserves, cuttings are unable to photosynthesize properly and die soon after insertion (John, 1979). According to Davis and Potter (in Haissig, 1982), photosynthesis may contribute sugar that is translocated to the base of the cutting. This increase in sugar availability at the base of the cutting also enchances root primordium development.

In some difficult-to-root species, the presence of leaves or needles for photosynthesis appears essential for rooting. In sycamore (Platanus occidentalis L.), dormant cuttings leafed out after being struck, probably because of stored food reserves. The cuttings then dropped their leaves within a short period of time and died (Land Jr., 1977; Foster and Thor, 1977). In conifers, active shoot growth and

foliage extension often occur simultaneously with rooting. Thus, there is competition for the available carbohydrates. Cameron and Rook (1974) showed that 75.0% of the photosynthates supplied to the roots of the cutting came from the old foliage. In Eucalyptus camaldulensis Dehn., the addition of auxins, sucrose, and nitrogenous compounds was not a sufficient substitute for the presence of leaves (Hartney, 1980).

Even though nutrient levels may be favourable, other physiological factors, such as the presence of root inhibitors, may depress rooting (Haissig, 1974b). Endogenous levels of root inhibitors increase with the maturation state of the donor (Davidson, 1974; Hartney, 1980). This increase in the concentration of the root inhibitor is directly correlated with a decrease in the ability of the cuttings to root (Paton et al., 1981).

Preconditioning of the Cutting or Donor Plant

Preconditioning can increase the rooting performance of the cutting by altering its physiological state at the time of striking. The storage of poplar cuttings in a refrigerator (2°C) induced callus formation and reduced the time between striking and rooting (Zsuffa, pers. comm.). Normally, cuttings from mature Sitka spruce (P. sitchensis (Bong.) Carr.) do not root; however, when they were placed in cool storage for up to three months, rooting success of 15.0 to 20.0% was achieved (McMullen, pers. comm.). Van den Driessche (1983) found that dormant Sitka spruce required ten weeks chilling at 2°C and Fraser fir (Abies fraseri (Porsh) Poir.) required eight weeks at 4°C to obtain the most rapid and complete rooting. In hybrid larch (Larix eurolepis (Henry)), cool storage (2°C) of dormant cuttings for six to nine weeks dramatically increased rooting, whereas warm storage (25°C) of more than three weeks resulted in good callus development, but significantly reduced subsequent rooting (John, 1979).

Ring-barking (girdling the stem prior to taking cuttings) contributes to rooting success. This technique is successful with some difficult-to-root species and with donors that have a more advanced state of maturation. In slash pine (P. elliottii Engelm.), up to 34.0% of the cuttings from girdled branches rooted, whereas a maximum of 3.0% rooted from ungirdled branches (Hare, 1978). Similarly, in sycamore, 100.0% of the treated branches rooted, but only 34.0% of the untreated ones rooted (Hare, 1976). This technique has made operational rooting of mature radiata pine feasible for seed orchard establishment.

It is important to minimize moisture stress at the time of striking cuttings. Most water is taken by cuttings through the cut base of the shoot and through the foliage in contact with moist soil. In Chamaecyparis obtusa (Sieb. a Zucc.) Endl. cuttings, the rate of water absorption decreased with time from when the cut was made (Toyuoka, 1977). A fresh cut at the base of the cutting at the time of striking resulted in a temporary increase in water absorption and in significantly higher rooting percentages. Substantially lower moisture deficits in the cuttings can be maintained if the foliage is sprayed with water (Cameron and Rook, 1974).

Removal of water soluble root inhibitors may be possible by soaking cuttings prior to striking. Good (in Bonga, 1977) showed that an inhibitor resembling abscisic acid could be removed from the foliage of Sitka spruce and European white birch (Betula pendula Roth.) by this method.

Season of Collection

The optimal collection times vary by species and conditions, but generally are best in early spring, just prior to the start of the growing season, and in late summer when growth extension is complete and lignification is just starting (Hare, 1978; John, 1979, Roulund, 1981). Under controlled growing conditions, however, collection periods can be extended. For example, when black spruce is grown under greenhouse conditions, cuttings can be taken throughout the year and successfully rooted, provided that supplemental lighting is available during the short winter days.

Exogenous Factors

Rooting Medium

The following table provides some examples of different media which can be successfully used.

Table 1. Examples of different media which can be successfully used

Rooting Medium	Species	Reference
Coarse gravel	Norway spruce	Kleinschmit et al. (1973)
Vermiculite and perlite	slash pine	Hare (1978)
Peat and sand	hybrid larch	John (1979)
Sphagnum peat and vermiculite	black spruce	Armson et al. (1980)
Sphagnum moss and coarse sand	several hardwoods	Tervonnen (1981)

The type of medium used influences the type of root system that develops. In Douglas fir (Pseudotuga menziesii (Mirb.) Franco), differences in root thickness, flexibility, and branching were observed among cuttings grown in different rooting media (Copes, 1977). When higher proportions of sphagnum peat were used, the root systems were finer and more branched than when smaller proportions were used. As the proportion of sand increased, the root systems that developed were longer, relatively unbranched, and less flexible. Similar results occurred in radiata pine (P. radiata D. Don) (Hill and Libby, 1969) and in white and

black spruce (Rauter, unpubl. data). Spruce cuttings grown in 100.0% sand not only had long, unbranched roots, but the roots also had a tendency to break at the base of the cutting when disturbed. If these cuttings were left through the winter dormant season and lifted in the spring prior to the onset of growth, their roots became flexible and easily manipulated (Rauter, unpubl. data).

The success of rooting and growth of roots is dependent upon the proper balance of aeration and moisture within the medium. Copes (1977) found that a medium with a high content of sphagnum peat was easily saturated, whereas one with pure perlite or perlite-sand required frequent watering and media with either too little or too much water produced poor rooting results. Rauter (unpubl. data) found that when the soil was saturated, sufficient aeration was not provided and the roots grew upwards through the rooting medium and into the air.

Ambient Temperature and Humidity

The few studies done on the success of rooting at varying temperatures have shown that cuttings can generally tolerate wide fluctuations. Rauter (1971) noted that even though spruce cuttings were exposed to temperatures ranging from 10°C to 32°C, there was no apparent detrimental effects on rooting. Werner (in Roulund, 1981) observed no damage to spruce cuttings exposed for short time periods to temperatures of 35°C to 40°C.

Ambient air temperatures can influence the length of time from striking to rooting. Ideal temperatures appear to be in the range of 20°C to 22°C for many species. As the temperature decreases, the length of the time required to root cuttings increases. Juvenile cuttings of black spruce that would normally have rooted within five to seven weeks remained green yet unrooted for four months due to low temperatures (Miller, pers. comm.).

Ideally, cuttings root well in cool moist air surrounding the tops and warm, solid conditions around the base. This temperature gradient allows greater activity in the base, while minimizing respiration and moisture stress on the top of the cutting. However, the benefit of heating cables is greatly reduced once the ambient temperature is 22°C or higher.

High humidity in the air surrounding the cuttings can reduce the stress caused by transpiration and thus promote rooting. However, high humidity when combined with high temperatures can promote the development of diseases such as Botrytis. It is also important to be able to maintain high humidity without saturating the rooting medium, particularly if the medium contains a high proportion of sphagnum peat. High humidity can be maintained by covering the cuttings with plastic sheets and shade cloth for greenhouse conditions (Armson et al., 1980) or aluminum-painted hoop-houses for nursery conditions.

Several types of misting equipment and control devices have been developed over the years to maintain humidity and humidity control. Many

have been tried in the Ontario program. The most reliable to date is one that can be set on a timer and adjusted for varying weather conditions.

Photoperiod and Light Intensity

During the spring and summer months, natural light intensity is sufficient for rooting. If cuttings are rooted during the short days of winter, it may be necessary to supplement the natural light. Supplemental lighting can also be used to induce bud formation and subsequent branch development in order to increase the number of available cuttings on donor plants. Phillion (pers. comm.) uses high-pressure sodium-vapour lamps to intensify the level of brightness to 500, 1000 and 2000 foot candles. The higher the light intensity, the greater the number of cuttings that are produced on juvenile scots pine donors.

Hormone and Chemical Application

Results of hormone and chemical application for improved rooting are contradictory. This can be partially attributed to the different experimental conditions, the different physiological states of the cuttings, and to our lack of understanding of some of the chemical and physiological processes occurring within the plant.

Although many recent studies have shown the beneficial effects of auxin application (Bhatnagar and Joshi, 1972; Hare, 1974; Morsink and Smith, 1974; Tervonnen, 1981), there has been little attempt to explain the nature of its effect. The enhancement of rooting may not be a result of the actual chemical applied, but perhaps a transformation of that chemical when absorbed by the cutting (Haissig, 1974a) or a physiological response of the cutting to the chemical (Haissig, 1982).

Both natural and synthetic auxins when applied to cuttings, usually increase the development of already existing root primordia (Haissig, 1974a). Thus, cuttings with initiated primordia would show better rooting percentages than those without preformed primordia. As an exception, recent work in jack pine has shown that a new synthetic chemical, an aryl ester of indole-3-butyric acid, has the ability to initiate root primordia (Haissig, 1979).

Chemicals have also been used to try to induce bud formation on donor plants. For example, a benzo-adenine purine spray applied to white spruce donors increased bud development significantly, but unfortunately, also created some toxic effects on the plant (Phillion, pers. comm.). Modification of concentrations and carriers used may reduce the toxic effect yet still be effective in promoting bud development.

Several of the above factors are closely interrelated. The alteration of one factor often necessitates the adjustment of others to retain rooting success. The large number of factors that influence rooting success also make it difficult to evaluate the effect of any one factor. Although modifications to improve rooting techniques are likely for years to come, standards have been established and these are being

successfully used to reproduce selected clones (Kleinschmit et al., 1973; Rauter, 1979).

BIOLOGICAL CONCERNS

Important problem areas are juvenility and maturation, early testing, genetic diversity, deployment strategies and clonal comparability. The following will highlight the current status of these problems as related to macropropagation.

Undue concern over biological problems has deterred the rapid development of clonal forestry, particularly with coniferous genera. To reiterate a statement of Libby (1984), 'we still need to know the answers to a lot of biological questions to do clonal forestry well, but they are no longer the sort of disqualifying questions that block further consideration.'. Many of these biological problems have been used by the traditionalists to oppose clonal forestry, rather than as a challenge to find solutions to the problems. Many satisfactory solutions to the various biological problems do exist, although they may not yet be the optimal solutions.

Juvenility and Maturation

One reason why vegetative propagation by rooting is not more widely used is our inability to sufficiently manipulate both ontogenetical and physiological ageing. We must be able to rejuvenate material that has an advanced state of maturation, otherwise selections for rooting programs must be made on young trees and we must accept the risks associated with early selection. These risks result from changes in growth rhythm and morphological characteristics as a tree shifts from the juvenile to the mature phase. This is of particular concern with species characterized by a large amount of free growth and only a moderate amount of predetermined growth, because selections may show a height advantage in the early years, but may not retain this advantage in later years (Eriksson, 1980). On the other hand, early selection can be effective for such traits as stem form and branch angle as there is little change in the expression of these over time.

Several ways have been developed to at least partially retard maturation and occasionally induce rejuvenation. Maturation can be retarded either by hedging the parent donor and not allowing upward growth (Libby and Hood, 1976; Hartney, 1980), by serial propagation of rooted cuttings (Kleinschmit, 1977; Morgan et al., 1980), or by using basal epicormic shoots which develop in many hardwood species (Hartney, 1980) and sprouting conifers (Libby, pers. comm.). In one instance, Dr. Ball of California produced some redwood propagules from tissue culture material derived from mature trees, and these propagules appear to be more juvenile than seedlings. Rejuvenation also seems to occur when scions from older trees are grafted and regrafted several times onto younger root stock (Franclet, 1981). All of these methods should improve the physiological state of the plant by shortening the internal transport system and improving the supply of water and nutrients to the periphery of the hedge,

cutting or graft (Fortanier and Jonkers, 1976). It appears that juvenility in plant tissue is dependent upon the distance of the tissue from the root system. As distances from the peripheries of the plant are shortened, there is also a reduction in the amount of root inhibitor substance present (Paton et al., 1981).

Fortanier and Jonkers (1976) found that hedging induces the formation of buds and tissues less mature than those being removed, thus inducing semi-ontogenetical rejuvenation. They emphasize that theoretically such rejuvenation cannot be repeated indefinitely because each pruning activates the meristems and thus stimulates ontogenetical ageing.

Increased maturation of the donor plant will continue to be a problem until more information is available on the physiological and biochemical processes of ageing. In the rooting of cuttings, not only is rooting percentage affected by the maturation state of the donor, but the speed and quality of rooting, subsequent growth rate, form and wood properties (Hood and Libby, 1978) and within-clone variation (Kleinschmit, 1977) are also affected.

Early Testing

Associated with the problem of maturation is that of early testing. Since much of the material can only be propagated in the juvenile state, test material must be evaluated early enough so that the donors can still be propagated with a degree of success and good future development. However, the earlier the evaluations are made, the greater the risk that these selections will not maintain their superiority at the end of their rotation. Kleinschmit (1977) states that this change in performance can be partially explained by differential gene activity over time. To minimize the risk of early evaluations and early selections, he suggests using adapted populations, maintaining a high genetic variation combined with repeated selection, selecting under typical field conditions and using close spacing in plantation establishment. The risk can also be reduced by testing full-sib families whose parents were superior performers or whose sibs already exist in older tests. Seed from the best families can be germinated and the resulting material used for clonal tests and mass propagation. If reliable relationships can be developed between growth potential and physiological and biochemical processes, such as photosynthetic efficiency, then the risks encountered through early selection may be overcome.

Genetic Diversity

Sufficient variation within any production program must be maintained to accomodate the wide variety of environmental conditions encountered by all commercially important species, even within a single seed zone or planting region. Thus, it is necesary to have a large number of clones in the clone bank, even though only a relatively small number may be in use in the production program at any given time. As some are eliminated from the production program, others must be added. Eventually, these new additions will come from advanced generation breeding

selections. There must also be close control on the number of propagules per clone that are produced for any production program. It is human nature to select only a few of the very best clones for production, however, this can lead to severe field losses. Vegetative propagation, as a production tool, could come into disfavour (Toda, 1974). Sweden and other countries have, or are contemplating, the establishment of rules controlling the number of clones and ramets per clone to ensure genetic diversity (Hedstrom and Krutzsch, 1983).

Deployment Strategies

When discussing deployment strategies, it is important to consider the size of the area under discussion. Large monoclonal plantations can be disasterous. In Japan, a single clone of cryptomeria, when planted on a small scale, did not show susceptibility to stem canker and foliage mites, but when planted over extensive areas, severe losses were incurred due to these pests (Toda, 1974). Monoclonal plantations may be lessserious with short-rotation species such as poplar. With short rotations, the pests do not have the same opportunity for a population build-up and there is less likelihood of abnormal weather conditions occurring. Even if serious losses do occur and the stand has to be removed, not as much time and money is lost when compared to a long-rotation species.

Small monoclonal plantations can be beneficial. Libby (1981) and Zobel (1982) recommended a mosaic of monoclonal stands rather than an intimate mixture of clones for a management system. When a small area (a few hectares or less) of single clones are planted, the variation within a plot is less than when a mixture of clones are planted. If individual clones fail, the problem can be readily recognized in the mosaic planting and these clones removed and replaced. This management method is more expensive because the identity of the clones must be maintained from collection through planting, yet greater gains can be made through better site utilization. If a program is sufficiently well-developed, then specific clones can be placed on specific sites (Zsuffa, pers. comm.). If there is a significant microsite variation, then a clonal mix similar to that proposed by Kleinschmit (1977) may be more realistic.

The number of clones to be used in a multiclonal mix is still under discussion. Initially, Japan favoured a large number of clones, expecting a larger gain than from seed orchard seed of the same parentage (Toda, 1974). However, when the performance of the clonal plantations was compared to these seedling plantations, the anticipated gain could not be confirmed due to the many silvicultural thinnings through the rotation. Libby (1981) recommends seven to thirty clones as a safe number in any single production plantation. The National Forestry Board in Sweden stipulated that a minimum of thirty to one hundred and twenty clones must be used depending upon the degree of testing for these clones (Twetman, pers. comm.). The number that should be used is affected by such variables as acceptable risk level, intensity of plantation management, spacing, plantation size and rotation age.

Since the production and establishment of many identified clones are more expensive than the production of a multiclonal mix, the extra costs have to be measured against the potential gains, potential risks and the most efficient use of the planting site.

Comparability of Clones

Before embarking on a large-scale clonal forestry program, there must be some assurance that material will maintain its superiority from the time of establishment until the time of harvest. For cuttings, Libby (1981) referred to this as the "comparability assumption". Shelbourne and Thulin (1974) found that the initial growth rate of radiata pine cuttings was slower than that of seedlings. Sweet and Wells (1974) had similar results in a nursery study for the same species. In the first experiment, cuttings were taken from six-year-old trees and in the second experiment, from variable age classes. Perhaps some of these slower growth rates may be attributed to the donors being more physiologically mature than the young seedlings. If cuttings had been taken from younger material or hedged material, the results might also have been different. Hood and Libby (1978) found that, initially, the more juvenile cuttings of radiata pine had faster relative height growth rates, but that the more mature cuttings had greater height growth rates than either the juvenile cuttings or seedlings of the same genotype. Roulund (1978) found that cuttings of Sitka spruce grew taller, showed less stem form variation, and had less variation in date of bud flush than seedlings. Although the initial stem form of the cuttings was poorer, within four years, the form was similar to that of seedlings.

WORK REQUIRED

Techniques are now available for rooting cuttings of most species. However, as with any new program being initiated, refinements must be made for the given set of conditions and species in use. Additional research in rooting procedures should have low priority compared to research in several other areas.

All of the biological problems mentioned in the preceding section require continuing research. The most pressing is the problem of controlling maturation. If this problem can be totally solved, mature selections can be effectively incorporated into a clonal program. This, in turn, will affect other areas, such as the need for early testing and thedevelopment of breeding strategies. In addition, many of the physiological and biochemical processes need to be better understood.

A neglected area of research is mechanization. Rooting is a labour intensive process. If several of the steps, such as the collection and setting of cuttings, could be mechanized, large-scale program costs would be reduced substantially. However, mechanization is only economical when the scale of operation is sufficiently large. Thus, we must first be committed to a clonal forestry program before we can justify the required expenditures on mechanization.

We also need to closely examine the breeding and deployment strategies being proposed. We must then set up a series of appropriate guidelines for various kinds of clonal forestry programs, thus avoiding problems in the future of our forests and opposition to clonal forestry generated by such problems from public interest groups.

CONCLUSION

Acceptance of new concepts is often difficult, particularly for those of us who have been trained in the traditional methods of practising tree improvement and forest genetics. However, the time has come to re-examine and re-evaluate many of the current programs, determine what they are capable of providing and then look at some different alternatives to see whether the results can be improved. One alternative that must be considered is the clonal option. I believe that for many species and in many programs across Canada, we will find that macropropagation and the clonal option will become a forestry reality.

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IN VITRO PROPAGATION OF CONIFERS

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ABSTRACT

Conifers are relatively easy to propagate by in vitro techniques from juvenile material. However, attempts to propagate mature conifers by in vitro methods have generally failed. We have obtained adventitious shoots, but no roots, in cultures of explants from mature Larix decidua and Pinus banksiana. In the Larix cultures, the shoots formed in slices of female cones collected during meiosis and in shoot tips collected during needle primordia initiation. In Pinus, the adventitious shoots were obtained from cultures of embryonic shoots excised from male buds at the time of microstrobili initiation. Some of the problems encountered in conifer tissue culture are discussed.

RÉSUMÉ

Il est relativement facile de propager les conifères par des techniques in vitro, à partir de matériel juvénile. Toutefois, les tentatives de propagation à partir de conifères d'âge mûr par ces méthodes ont généralement échoué. On a réussi à obtenir des pousses adventives, mais pas de racines, dans des cultures de boutures de Larix decidua et de Pinus banksiana d'âge mûr. Les pousses obtenues dans les cultures de Larix provenaient de tranches de cônes femelles prélevées lors de la méiose et aussi de bouts de pousses prélevées au début de la formation des rudiments (primordia) des aiguilles. Dans les cultures de Pinus, les pousses provenaient de pousses sexuelles males prélevées durant la formation des strobiles. L'auteur traite de certains problèmes propres à la culture des tissues de conifères.

INTRODUCTION

In vitro cloning techniques have reached a level of sophistication where large-scale propagation is now possible for several hardwood species (for forest trees, see Brown and Sommer, 1982; Mascarenhas et al., 1982; for fruit trees, see Hutchinson, 1982; Mascarenhas et al., 1982). Although some hardwood species (Spiegel-Roy et al., 1980; Mascarenhas et al., 1982) can be propagated from mature specimens, most can still only be propagated from juvenile material. Similarly, conifers, except Sequoia (Ball et al., 1978), can presently be

propagated from juvenile material only (David, 1982). Although *in vitro* propagation of juvenile material can be useful (i.e. for rapid clonal production of plantlets from valuable embryos obtained in controlled crosses (Mott, 1981; Smith *et al.*, 1981)), the main requirement for forest improvement is propagation of selected mature specimens (Zobel, 1981).

IN VITRO PROPAGATION OF MATURE CONIFERS

The difficulties in propagating explants from mature trees arise as a result of the lack of organogenesis *in vitro*. Two reasons can be suggested for this: (1) there are no organogenetically competent cells in the explants; and (2) there are a few competent cells but these are prevented from entering organogenesis by correlative inhibitions imposed by neighbouring tissues (Henshaw *et al.*, 1982). These conditions may not be static. There are moments in the annual growth cycle of the tree when tissues programed to follow one type of development are switched into a different development plan. Examples of this are initiation of primordia, the change from sporophyte to gametophyte, and stomatal differentiation. It is possible that during such developmental changes, some cells temporarily become more organogenetically competent, or if already competent, are temporarily released from correlative inhibitions (Bonga, 1982b; Riding and Aitken, 1982). This possibility of a temporary improvement in organogenetic capacity at specific moments at specific sites in the tree has provided the impetus for much of our work over the last few years. So far, we have found three developmental stages in the annual growth cycle during which, at least in some trees, explants capable of adventitious shoot formation can be obtained. These three stages are female cones during meiosis, male shoots during microstrobili initiation, and vegetative shoots during needle initiation.

Female Cones During Meiosis

A few small adventitious buds were obtained from somatic tissues of immature female cones of *Pinus mugo* (Bonga, 1981). More success was achieved with the somatic tissues of female cones of one twenty-five-year-old *Larix decidua* tree. In the spring of 1981, female cones were collected from this tree, cut in transverse slices, and cultured. Slices of cones collected at about the time of meiosis produced adventitious shoots; slices of cones collected earlier or later did not (Bonga, 1982a).

This experiment was repeated the next year and again only the collections that contained cells in meiosis (all stages between megaspore mother cell and tetrad) were responsive. However, the origin of the adventitious shoots was different in the second experiment. These shoots, instead of arising from slices of the stalk of the cone, developed from slices containing the bracts and scales. They arose mainly from the cut surfaces of the young bracts (Fig. 1) but occasionally from the side of the bract. These shoots were separated from the bracts and subcultured. In subculture, the shoots produced callus at their base. This callus formed new adventitius shoots (Fig. 2), which could be separated and subcultured individually after a few weeks. In one of the adventitious

FIGURES

Figures 1-4 *In vitro* culture of slices of immature female cones of *Larix decidua*.

Figure 1 Adventitious shoots (s) are forming on the cut surface of a swollen cone bract (b). x 15.

Figure 2 Adventitious shoots are developing on callus formed by the adventitious shoots shown in Figure 1. x 6.

Figure 3 A needle of an adventitious shoot on a bract has swollen and is forming numerous bumps (b). x 15.

Figure 4 The bumps shown in Figure 3 have turned into numerous shoots. x 6.

Figures 5-6 *In vitro* culture of male shoots of *Pinus banksiana* collected when the strobili were primordial.

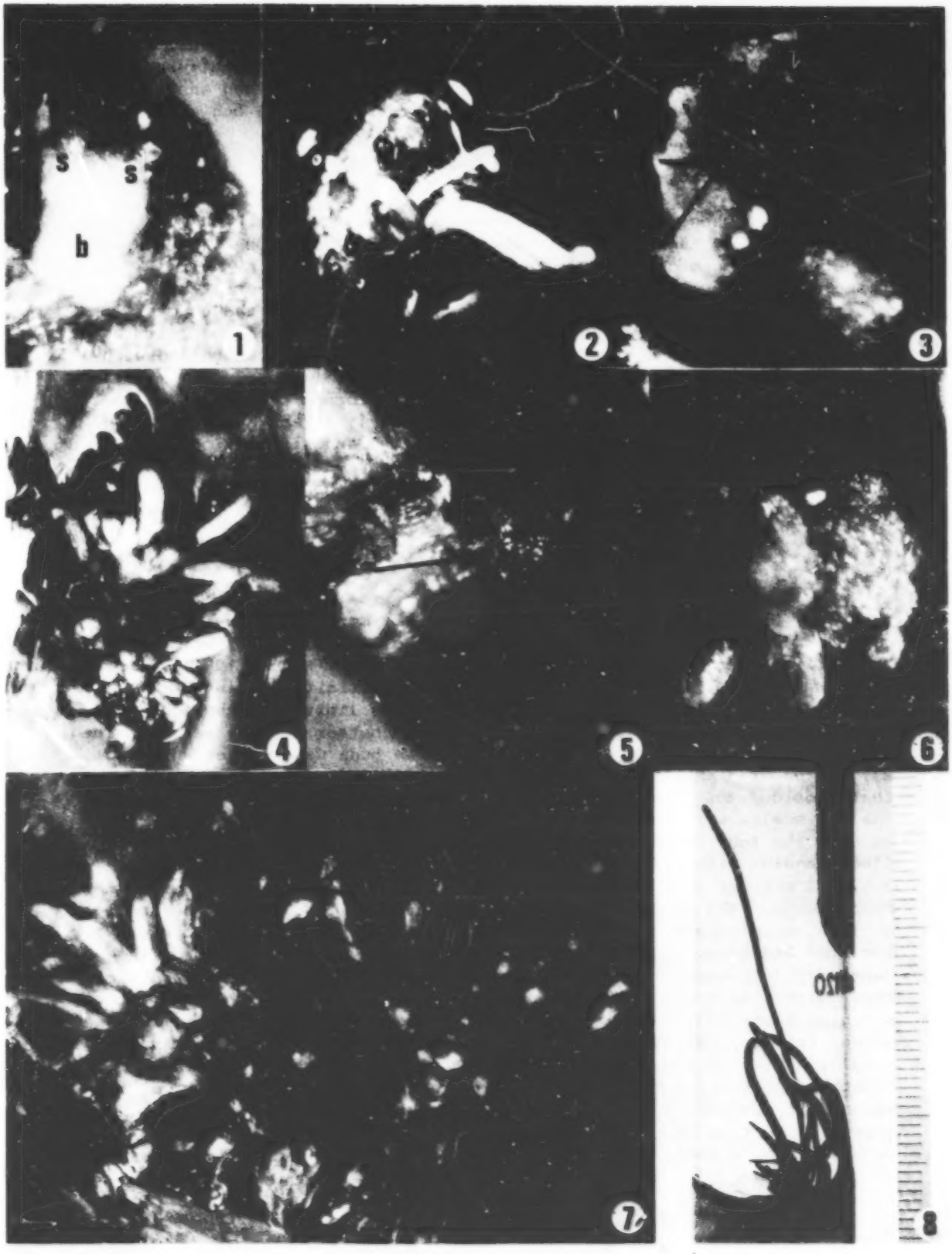
Figure 5 The shoot has produced a callus which is forming a few adventitious shoots (arrow). x 15.

Figure 6 Elongated needles of one of the adventitious shoots shown in Figure 5. x 15.

Figures 7-8 *In vitro* culture of vegetative shoots of *Larix decidua* collected at the time of needle primordia initiation.

Figure 7 The shoot has produced callus which is producing numerous adventitious shoots. x 8.

Figure 8 A shoot removed from the mass of shoots shown in Figure 7 and subcultured. One needle in this shoot reached a length of 43 mm. Scale on the right hand side is in millimetres.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shoots arising from the bracts, the needles started to swell. Subsequently, small bumps developed along the needle surface, each of these forming a small shoot (Figs. 3 and 4). In this experiment, a total of thirty-six adventitious shoots survived excision and subculture. Their needles elongated to mature length, but their stems remained short and roots were not formed. Most shoots had a distinct apical bud and occasionally one or two lateral buds.

Shoots During Microstrobili Initiation

Branches with male buds were collected from one Pinus banksiana tree on July 28, August 11, September 13 and 17, 1982. On July 28th and August 11th, the microstrobili in these buds were still in their primordial state, but by September 13th, they had differentiated into distinct microstrobili. The bud scales were removed and the shoots with the primordial or more advanced microstrobili were excised and cultured on Litvay et al. (1981) medium with all minerals at half strength and with 1 mg/L IBA and 2 mg/L BAP, at 21°C under $50_u Em^{-2}$ sec^{-1} Gro-Lux illumination for sixteen hours daily. Most of the cultures formed callus but no adventitious organs. In the cultures of the July 28 and August 11 material, three (out of one hundred and twenty) and six (out of eighty) cultures, respectively, produced callus that formed small shoots (Fig. 5). These shoots were excised and transferred to hormone-free medium where the needles of some of the shoots elongated a few millimetres (Fig. 6).

Vegetative Shoots During Needle Formation

Shoot cultures of Abies balsamea are morphogenetically most active if the shoots are excised from buds collected shortly before bud break, i.e. when laterial bud primordia are formed (Bonga, 1981), or from late summer buds, i.e. when needle primordia are initiated (unpubl. data). In these experiments, morphogenesis was limited to the formation of small, abnormal adventitious shoots. Much better results were obtained with Larix decidua. Branches with vegetative buds were collected August 23, September 3 and 18, 1982, and January 10, 1983, from the same tree that provided the female cones for the experiment previously mentioned. The bud scales were removed and the shoots (two hundred and forty from each of the four collections) were excised and cultured on Litvay et al. (1981) medium with all minerals at half strength and with 0.1 mg/L IBA and 2.0 mg/L BAP, at 21°C under $50_u Em^{-2}$ sec^{-1} Gro-Lux illumination for sixteen hours daily. The shoots from the August 23rd collection were too small to survive excision and culture. Of the September 3rd collection, only a few survived and grew. The best results were obtained with those from the September 18th collection. The embryonic shoots and needle primordia of this collection commenced elongating after a few days in culture. After reaching a few millimetres in length, the needle bases started to form a green, friable, callus that soon covered the stem between the expanding needles. Some of the callus surface was granular, but most of it was smooth. About six weeks after the cultures had been started, large numbers of adventitious shoots developed from the smooth, but not the granular, surface of the callus (Fig. 7). This happened in sixty of the two hundred and forty explants. As the callus increased in size, most of

its interior cells, as well as most of the original shoot disintegrated, leaving a large hollow in the callus. The needles of the adventitious shoots on the callus elongated. After they reached a length of 2.0 to 4.0 mm, the callus with adventitious shoots was cut into several pieces and each was transferred to fresh nutrient medium without growth regulators. In subculture, the needles of the shoots elongated further. The subcultured smooth callus maintained a high growth rate and formed more adventitious shoots on its surface. A few new shoots also formed from lateral buds of the adventitious shoots, but most new shoots developed directly from the smooth callus. The growth rate of the callus, and the rate of new shoot formation was rapid enough to allow separation of shoots (with some callus attached to the base) about once every two weeks. In five months, the subcultures produced three hundred and forty adventitious shoots or clumps of shoots. The needles of these shoots grew rapidly to about mature length, or sometimes much longer (up to 43.0 mm) (Fig. 8). After the needles started elongating, apical and occasionally a few lateral buds became visible in many of the shoots. None of the stems of the adventitious shoots elongated to more than a few millimetres and roots were not formed.

The shoots excised from dormant buds of the January 10th collection showed little stem but normal needle elongation. Some granular callus was formed, but no adventitious shoots developed from the callus.

DISCUSSION

The above experiments show that explants of at least a few conifer trees can produce adventitious shoots *in vitro*. The experimental results also strengthen the view that there is a close association between organogenetic competence and specific developmental events in the tree. The earlier-posed question, whether the increased morphogenetic ability is caused by a temporary increased competence of a few cells, or whether competent cells are temporarily freed from correlative inhibition, is still unanswered.

It has often been found that adventitious shoots arising from tissues of mature trees are juvenile. This applies to shoots arising from tissues of several species *in vitro* (i.e. *Vitis* (Mullins *et al.*, 1979), and *Hedera* (Hackett, 1980)), and is also common in adventitious shoots arising *in vivo* (i.e. from nucelli, and sphaeroblasts (Wellensiek, 1952; Hackett, 1980)). However, there are exceptions as indicated by the following examples. Some apple cultivars which were regenerated *in vitro* were not completely juvenile (Sriskandarajah *et al.*, 1982). Plantlets produced from shoots of *Sequoia sempervirens* from the upper part of the tree (Ball *et al.*, 1978; Franclet, 1980) and from cotyledons of *Pseudotsuga menziesii* (Mapes *et al.*, 1981) were plagiotropic. Some hardwood species produced plantlets *in vitro* that flowered precociously (Huhtinen, 1976; Mascarenhas *et al.*, 1982). These observations could mean that rejuvenation during adventitious shoot induction is sometimes incomplete, or that adventitious shoots sometimes mature rapidly. Such rapid maturation could in part be due to environmental stress in culture. Stress often results in early flowering (Longman, 1976), which is

indicative of rapid maturation. Some retention or re-establishment of a mature type of physiology could be one reason why the adventitious shoots from callus derived from explants of mature Larix decidua did not elongate and root.

Occasionally, juvenility will increase if adventitious shoots are repropagated in vitro through several generations (Mullins et al., 1979; Franclet, 1980, 1981). In our cultures of Larix decidua, successive subculturing did not improve stem elongation and rooting.

Over the years, we have tried to obtain adventitious shoot formation from explants of mature trees of several conifer species. So far, the best response has been with female cones and vegetative shoots obtained from one Larix decidua tree. Similar explants from a few other nearby Larix decidua trees, of approximately the same age, produced no adventitious shoots in culture. This suggests that the Larix decidua tree that produced the adventitious shoots may have a higher genetic propensity for organogenesis than the other trees. Genotypic differences in organogenetic ability have been noticed in cultures of several conifers (Brown and Sommer, 1977; Arnold, 1982) and other plants. In studies of the genetics of organogenesis, it has been found that hybrids often have a higher organogenetic capacity than inbred lines (Reisch and Bingham, 1980; Alicchio et al., 1982). It has been suggested that to obtain improved regeneration, genes that favour organogenesis could be transferred by breeding into plants lacking these genes (Reisch and Bingham, 1980).

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DEVELOPMENT OF GENETIC STRATEGIES FOR CLONAL FORESTRY

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ABSTRACT

Clonal forestry promises numerous benefits for forest management. The "clonal option" also offers numerous challenges to forest geneticists who must design the clonal forestry breeding strategies. The current genetic strategies developed around open-pollinated seed orchard programs to exploit additive genetic variance will not generally be appropriate for clonal programs wanting to exploit non-additive genetic variance. Certain crop breeding programs and principles can serve as a useful guide in redesigning our tree breeding strategies. Population structure and function, selection, mating, testing, and deployment are some of the operational aspects whose genetic components deserve careful scrutiny. Some "fine-tuning" of the genetic strategies in these areas will be necessary in order to take full advantage of the possible benefits.

RÉSUMÉ

L'utilisation des clones recèle de nombreuses promesses pour l'aménagement forestier. Elle pose aussi de nombreux défis aux généticiens, qui doivent concevoir des stratégies clonales adaptées d'amélioration des arbres forestiers. Les stratégies génétiques actuelles, qui reposent sur les vergers à grains resultant de la pollinisation libre, ont été développées pour tirer parti de la variance génétique additive. Elles ne conviendront généralement pas aux programmes de clonage orientés vers l'exploitation de la variance non additive. Certains programmes et principes d'amélioration des cultures peuvent guider utilement l'élaboration de nos nouvelles stratégies d'amélioration des arbres. La structure et la fonction des populations, la sélection, les croisements, les tests et la mise en place sont certains des aspects opérationnels dont les éléments génétiques doivent être soigneusement étudiés. Le réglage fin des stratégies génétiques dans ces domaines sera nécessaire si on veut tirer parti de tous les avantages possibles.

INTRODUCTION

At a recent symposium on "Research Needs in Tree Breeding" (Guries and Kang, 1980), a number of speakers stressed the need for better

information about appropriate breeding strategies and, in particular, strategies that incorporated the use of vegetative propagules. In his keynote address, Libby (1984) identified a number of operational areas, such as managing populations, that will require rethinking and redesigning to effectively use clonal material. He also briefly mentioned a few genetic consequences that should be considered. This paper will address the question: in what areas and to what extent might we need to redesign our genetic strategies?

In order to gain a proper perspective, I will first review the current status of tree breeding programs, and compare certain aspects of these programs to crop breeding programs. I will then discuss specific genetic considerations for designing clonal breeding strategies.

CURRENT STATUS OF TREE IMPROVEMENT PROGRAMS

Past and current tree breeding programs have not been formulated principally on what is most genetically efficient, but rather on what is genetically possible under a given set of biological (non-genetic), logistical, and socio-economic constraints. Conifer programs have progressed over the years from simple mass selection programs (collecting seed from the best parents of the best sources) to complex clonal orchards with their associated, statistically complicated, multi-function progeny tests. Since most of these programs were initiated after the advent of theoretical quantitative genetics, tree breeders recognized that the strategies they used could exploit only part of the genetic variation. Borrowing breeding programs from other crop plants did not look promising because of the long-lived nature of trees. The notion of biclonal orchards to exploit outstanding special crosses was considered in a number of programs, but because of genetic and logistical problems, biclonal orchards have not become widespread. Vegetative propagation was recognized as a better method of exploiting all of the genetic variation, but biological problems have, to date, prevented its widespread use in most of the important conifers. At the same time, some hardwood breeders have taken considerable strides toward clonal forestry.

The relative ease with which certain hardwoods could be propagated by cuttings, together with the great variability exposed when inter-specific crosses were made, led to a strong clonal program (eg. Salicaceae). However, the easy, short-term gains readily available in these programs, in many cases, inhibited the development of sound, long-term genetic programs (Zobel, 1982; Mohrdiek, 1983). Therefore, it appears that conifer and hardwood breeders have reached the same conclusion, although from different directions, that present breeding programs must be carefully examined and redesigned where appropriate.

PERSPECTIVE FROM PLANT BREEDING

At this "cross-road" in the development of tree breeding programs, it is appropriate to again look at genetic management systems of the crop breeders. We cannot reasonably expect to adopt specific crop

breeding plans for tree breeding because agronomic crops and forest trees have different evolutionary backgrounds and domestication histories (Burdon,1983; Silen, 1982). However, since crop species have gone through many more generations of breeding and breeders have considered some of the same questions we are now or will be asking, we may learn some valuable lessons regarding the exploitation of genetic variances and the tailoring of selection methods to the mating system.

Although plant breeding systems can be described in a number of ways, Simmonds (1979) classifications are most pertinent to this discussion. Four crop breeding systems, namely 1) inbred lines (IBL); 2) hybrid varieties (HYB); 3) open-pollinated populations (OPP); and 4) clones (CLO), are recognized, each designated primarily by the genetic structure of the production (i.e. the harvested) population. These classifications are not totally exclusive and some ambiguity between populations is possible; in general, however, they serve well.

The first important distinction among the four classifications is the difference in opportunity each provides for the breeder to exploit portions of genetic variation. Clones and hybrids can completely exploit all genetic variation including overdominance (both real and pseudo), and inbred pure lines can use all but overdominance. Open-pollinated populations can also utilize all of the genetic variation but the non-additive portion is expressed in an inefficient hit-or-miss manner due to the accompanying heterogeneity of the population (Simmonds, 1979). How do our tree breeding programs compare?

One can easily recognize that our typical seed orchard programs are a particular kind of OPP, referred to in a crop breeding context as a "synthetic" (SYN). Hardwood programs that utilize rooted cuttings as propagules are easily classified as belonging to CLO. (Hybrid poplar breeding, although it appears to contain elements of both HYB and CLO, is more appropriately classified as a clonal program because the production plantings are established with clones.) To increase genetic gain, tree breeders who are already maximally exploiting their OPP, must move towards one of the other systems. At present, the obvious choice is clonal propagation.

A second distinction among the four classifications is that they have been designed, in general, around the mating system and life cycle of the species. IBL programs involve inbreeding annuals propagated by seed; OPP programs are usually used for outcrossing perennials propagated by seed; and CLO programs involve outcrossing perennials propagated vegetatively (Simmonds, 1979). Crop breeders have developed strategies for fully exploiting genetic variation in ways consistent with the mating system of their plants. Tree breeders can benefit, not by lifting entire programs from plant breeding, but by adopting the best elements in each program consistent with the mating system of forest trees (i.e. outcrossing).

The dividing lines between the four systems are not rigid. During the developmental phase, OPP and CLO will be parallel, complimentary operations until all regeneration requirements can be met by

clones. Elements of OPP breeding will always be retained for long-term breeding, and in the future, even elements of the other two systems will be included given that certain physiological problems are solved.

In the following, I would like to point out four additional lessons that can be learned from crop breeding.

The first is in regard to the "mythology" that has sprung up around hybrid corn. No one will argue that hybrid corn has not been a great success. The hybrid corn technique has even been proposed for forest trees. However, hybrid corn is only a convenient and clever way of packaging genes and selling them to farmers. There is growing evidence that other methods may have been equally effective in producing the gains (Hallauer and Miranda,1981). For example, if corn could be clonally mass produced, there would not be any need for hybrid programs because the clonally-reproduced upper fraction of a recurrent-selected, open-pollinated population would be phenotypically equivalent for yield to the hybrids produced from the population (Simmonds, 1979). However, economic theory has played a greater role in the direction and scope of corn breeding than genetic theory.

Second, and related to the first point, concerns the increasing emphasis being placed on recurrent selection programs for intrapopulation improvement. There appears to be growing concern among crop breeders regarding the stock populations from which the breeding lines are derived. The direction most plant breeding programs are currently taking is towards increasing the frequencies of favourable alleles in order to meet the long-term goal of better extracted lines (Hallauer, 1981). While hybrid corn is likely to be on the scene in the foreseeable future, hybrid seed will remain a convenient way of packaging genes, and the "real" breeding work will be done within populations.

The third point is that there is a trend in other programs involving perennials towards the increasing use of clones. Among these species, we find rubber, tea, coffee, and a number of other tropical crops (Simmonds, 1979). As plant biochemistry and physiology become more advanced, we can expect to see the trend involve other species. The interpretations of this trend will be many and varied. Many breeders already see the advantages of clones and are willing to risk a change of program.

The final point is to recognize that breeding strategies should be specific to overall intensities of management. So far, we have been discussing breeding strategies geared to the highly-developed agronomic systems of western countries. High yielding, inbred pure lines and hybrid varieties have been developed to compliment and enhance advances in our farming systems where a high degree of site modification is practiced. Breeding of improved stock for developing countries with basic agricultural practices, at centers such as CYMMIT in Mexico, takes a different approach (Borlaug, 1983). The main objectives of these programs are to produce broadly-adapted populations with high genetic buffering to withstand a great variety of conditions and still produce satisfactory yields. Tree breeding programs generally have similar objectives and

caution should be exercised in selecting too intensively for specific traits and site requirements, particularly while our plantation sites are relatively primitive and our ability to alter them is limited (Silen, 1982).

SPECIFIC GENETIC CONSIDERATIONS FOR CLONAL BREEDING STRATEGIES

To Redesign or not to Redesign?

One question that may arise at this point is: "Why worry about redesigning breeding strategies until we know more about the modes of inheritance - the relative importance of additive and non-additive effects?" I have three answers to this question.

First, I suspect the questioner may propose to find this information on additive and non-additive effects from ANOVA derived variance component estimates. There is mounting evidence that, in a given experiment, these estimates can be inaccurate and on occasion totally misleading, particularly in the realm of detecting epistatic effects (Jana, 1972; Gilbert, 1973; Konig, 1982). However, there are accumulated results from a number of studies which indicate that the dominance component is significant (Wilcox, 1976; McKeand, 1981; Libby, 1983; Shaw, pers. comm.).

Second, and consistent with the first answer, even if we totally disregard ANOVA estimates, there is indirect evidence that non-additive effects are present from the severity of inbreeding depression exhibited by forest trees. Another piece of indirect evidence is the often observed disparity between clonal repeatabilities and narrow-sense heritabilities.

Third, even if there is little or no non-additive genetic variance, there are still genetic advantages to using clones (Libby, 1984), and all of the non-genetic advantages remain.

Given that tree breeders should proceed with clonal forestry equipped with the current, best genetic information, we face the question originally posed in the introduction: in what areas and to what extent might we need to redesign our genetic strategies?

Even though operational aspects may require some major changes, the basic breeding programs will not, at least on a large scale. The primary objectives of selection for broad adaptability together with selection for specific, economically important traits, will remain foremost. Tree breeding has been described as a sequence of operations involving selection and packaging of genes using native and subsequent derived populations as "raw material" (Libby, 1973). Some "fine tuning" of the genetic components of these operations is necessary. The particular components I will discuss in the following sections are population structure and function, selection, recombination (mating), testing and deployment. Although these components are intricately related, I will treat them separately in order to simplify the discussion.

Population Structure and Function

Classification by function is a useful conceptual framework for discussing populations. The four most frequently mentioned populations in tree improvement literature are: propagation population; genetic test population; breeding population; and wood production population. Other functional populations have been suggested and these are discussed more extensively by Kang (1982). These functional populations are kept physically distinct in most breeding programs, although this is not always necessary.

Propagation Population

The propagation population in a seedling strategy is, of course, the seed orchard. Under a clonal strategy, this population could differ radically from its seed orchard counterpart in both physical appearance and genetic constitution. In many clonal programs, this population will consist of a number of closely-spaced hedged trees.

The genetic diversity of the propagation population will differ, under the clonal option, from the diversity in a seed orchard. For a special site problem, the propagation population could conceivably consist of a single clone; for a large plantation covering diverse sites, twenty clones; and for a regional program, two hundred clones. Yet, all these clones could be kept in one hedge-orchard. Therefore, care must be taken to distinguish between a cutting or hedge orchard and a particular propagation population when considering population size. Such a high level of built-in flexibility cannot be easily reached in an open-pollinated seed orchard program, which usually has fewer than fifty clones after roguing.

Although I appear to be equating population size to genetic diversity, I am not; I assume that effective coancestry controls and other measures to keep diversity high will be practiced. The genetic implication of this flexibility is that the breeder can more accurately match genotypes to site. Management advantages of cutting orchards over seed orchards are numerous, some of which were mentioned by Rauter and Hood (1982) and Libby (1984).

Genetic Test Population

In most seed orchard programs, a single genetic test serves a multiplicity of purposes; the two main ones being evaluation of parental breeding values, and provision of new raw material for advanced generation selection (McKinley, 1983). If a clonal program, rather than a seed orchard program, is adopted, the progeny test function (i.e. backwards selection) will become even less important than the selection function. The main change will be a trend away from large, multipurpose tests towards a number of different test populations of varying numbers of families, clones and ramets. Each test will serve a single specific function. The overall testing effort may increase relative to that required by the seed orchard option; the increase will be proportional to the intensity of genotype-site matching.

Breeding Population

In a seed orchard program, this functional population is often physically the same as the propagation population, although they can be separate. In a clonal program, the breeding population and the propagation population will almost always be separate entities. Usually the maturation state of the cutting donor will not be conducive to flowering.

The size of the breeding population may be the most important difference between clonal and seedling programs, especially if dominance or epistatic effects are important. If indeed these effects are important, then it is critical that large, effective population sizes be maintained in order to retard random loss of favorable dominant alleles and favorable epistatic combinations (Namkoong, 1982).

First proposed by Baker and Curnow (1969), the idea of subdividing the breeding population into sub-populations has been readily adopted by tree breeders (IUFRO, 1976; vanBuijtenen and Lowe, 1979; Kang, 1980). Depending on how the populations are managed and overall program organization, the method is either referred to as sublining (single population) or multiple population breeding. Either method will allow the breeder to exploit dominance and epistatic effects if these are important (Namkoong, 1982). Multiple population breeding is, however, more consistent with the concept of selecting clones for very specific uses.

Wood Production Population

These populations represent our future plantations. They contain a very large number of genotypes when derived from open-pollinated seed orchard seed, many related as half- or full-sibs. A clonal plantation will consist of a much smaller number of genotypes, yet it is possible to make it at least, and probably more so, as diverse as a seedling plantation due to the greater amount of genetic control possible (Libby, 1984). Particular sets of clones that perform well together may be allocated in an advanced clonal program rather than a random assortment of clones from an appropriate pool.

Native Populations

The original or founder population will be the least affected by the changeover from seedling to clonal forestry. However, techniques of clonal forestry, perhaps by in vitro methods yet to be developed, may enhance our ability to conserve some of the original variability for the future.

Sampling the original population (euphemistically referred to as plus tree selection) could conceivably be different for clonal programs than for seed orchard programs, with greater attention being paid to micro-site adaptation of individual genotypes. Such would be the case if these original genotypes could be vegetatively reproduced.

Selection

Selection is the process that converts a test population into a breeding population and propagation population. Although in practice selection follows mating, the breeder should consider them in reverse order when designing a strategy. If a method of selection has been chosen that is appropriate to the program's objectives and resources (e.g. one or some combination of mass, family, within family, combined or index), the population structure which is necessary to carry out the selection should be evident. This required population structure in turn limits the choice of mating design.

The overall objective within the continuum of breeding populations is recurrent selection for increasing the frequency of favorable alleles and/or allelic combinations. This objective is common to both seedling and clonal programs. However, the method of capturing all or part of the genetic variability exposed during the process (the so-called "packaging" operation) is, of course, quite different as is the effectiveness. A seed orchard program is equivalent to "packaging the (recombined genes from the) selections", while a clonal program is equivalent to "selecting the (gene) packages"; the latter operation is more effective.

The use of clones can increase the efficiency of selection, but depending on allocation of resources there can be a trade-off between selection intensity and better estimates of genotypic values (Libby, 1969). For overall population improvement, relatively few ramets and large numbers of families and clones would be most effective for long-term objectives (Simmonds, 1979). The same principle applies to selection among clones for production in the early cycles.

Recombination (Mating)

The question of mating designs has occupied the attention of tree breeders for nearly two decades (e.g. Namkoong et al., 1966, vanBuijtenen and Namkoong, 1983). The choice of mating design depends on the type of genetic structure desired in the resulting progeny (genetic test population). A single mating design is often not the best when there are a number of different objectives, as there are in both clonal and seedling programs. In the clonal program, however, there is a clearer dicotomy of functions. The first is advanced generation selection for population improvement; the second is selection among a large number of genotypes for vegetative propagation. The first function is best served by the least amount of work that is possible consistent with the goal of keeping the effective population size large (i.e. single pair matings) (Libby, 1983). The second function is best served by the greatest amount of work you can afford which, in most cases, is not much. However, one of the partial diallel designs would be satisfactory (vanBuijtenen and Namkoong, 1983).

Whatever the choice of mating design, the overall breeding program should endeavour to keep inbreeding under control. Therefore, pedigreed families are a must, or a strict sublining program should be

followed. Inbreeding in the breeding population can be tolerated. In a clonal program, it can be more effectively manipulated to yield greater gains. Inbreeding in the production population, whether clones or seedlings, is to be avoided.

Testing

For this discussion, it is useful to distinguish between selection and testing. Testing is the evaluation of phenotypes in a suitable environment. Selection is the application of the evaluated data and genetic theory to choose the genotypes for breeding or propagation. They are certainly intimately related, but there are some distinct issues involved.

The above contrast between current and proposed genetic test population has its counterpart here; single, large multipurpose tests will give way to more and smaller test plantings. These smaller test plantings will be designed to match specific clones to specific sites and to test for very broad adaptability; the former is likely to come later in the program. This testing hierarchy will allow breeders to identify and deal with G X E interactions much more efficiently than is possible with seedlings.

Experimental designs that are appropriate for testing large numbers of entries, such as incomplete block designs, will probably replace randomized complete blocks in the early stages of that testing program. Investigation into designs that can adequately handle the heterogeneity of most of our forest sites will be necessary.

Deployment

In the case of seedling forestry, bulked seed from a given seed orchard is allocated to a large number of sites (consistent with site classification, similarity to test environment, etc.). The idea is to serve all purposes with a single, variable, "synthetic" population. On the other hand, in "clonal forestry", special clones can be sent to specific sites, and general purpose clones can be sent to unclassified sites. Other deployment advantages have been presented by Libby (1977) and Zobel (1982).

Influence of Advances in Molecular Biology

The potential of molecular biology has been previously addressed by Libby (1984). Notwithstanding the justified caveats, I wish to briefly mention some of these techniques as possible new sources for variation that can be exploited by vegetative propagation.

Haploid and dihaploid culture is progressing successfully in a number of tree species. The major potential advantages of this would be to allow the breeder to directly estimate the breeding value (Simmonds, 1979) and to unlock another level of genetic variation--segregating individual gametes.

Protoplast or cell fusion has the potential to create large amounts of new variation (Ahuja, 1982) and the somaclonal variation arising from cultures may also be an important source of new variation (Larkin and Snowcroft, 1981). However, it is likely that only a limited amount of this variation will ever be usable and only after very extensive testing.

CONCLUSIONS

Clonal forestry offers many advantages of both a genetic and silvicultural nature. The decision to use the clonal option will potentially effect all the genetic components of a breeding strategy. Modifications to these components will be needed if the full advantage of clones is to be realized. In most instances, the alterations necessary are of degree and not kind.

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REJUVENATION: THEORY AND PRACTICAL EXPERIENCES IN CLONAL SILVICULTURE

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ABSTRACT

As a result of rejuvenation, clonal silviculture is in full expansion around the world as demonstrated by recent industrial clonal reforestation in the Congo and Brazil.

After a brief review of the main concepts and theories to explain the phenomena of maturation and rejuvenation, these concepts will be illustrated with examples from AFOCEL and associated laboratories.

At AFOCEL, emphasis is placed on the rejuvenation of Eucalyptus among broadleaved species, and Sequoia among conifers. In addition, the most efficient methods to rejuvenate aspen, cherry, sycamore, walnut, Douglas fir, giant Sequoia, Maritime pine and Norway spruce are discussed.

Hedging, cutting back, grafting, and rooting of cuttings, used once or repeatedly and combined with each other or with chemical treatments, such as the foliar application of BAP, are used to rejuvenate clones. When enough juvenility is recovered, the work is then pursued in vitro by shoot and meristem culture.

Plantlets which are completely, or sufficiently, juvenile are produced to be used as cutting donor plants in commercial nurseries for classical cloning. The systematical use of biochemical tracers of juvenility, such as the total peroxydasic test, could provide stimulation for progress in this field.

RÉSUMÉ

Grâce à la rejuvénilisation, la sylviculture par clones est en pleins expansion dans le monde, comme le démontrent les efforts industriels récents de reboiement à l'aide de clones au Congo et au Brésil.

Après un bref exposé des principaux concepts et théories concernant la maturation et la rejuvénilisation, je présenterai des exemples tirés des travaux effectués à AFOCEL et dans les laboratoires associés.

À AFOCEL, on met l'accent sur la rejuvénilisation d'Eucalyptus chez les espèces à larges feuilles et de Sequoia chez les conifères. J'examinerai les méthodes les plus efficaces de rejuvénilisation du peuplier (tremble), du cerisier, du platane occidental, du noyer, du douglas taxifolié, du Sequoia géant, du pin maritime et de l'épinette de Norvège.

Des techniques d'émondage en haie, de recépage, de greffage et d'enracinage des boutures, réalisées une fois ou répétées, et combinées ou non entre elles et à des traitements chimiques, comme l'application de BAP sur les feuilles, sont employées pour la rejuvénilisation des clones. Lorsqu'une juvénilité suffisante est retrouvée, le travail est ensuite repris in vitro par la culture de pousses et de méristèmes.

Des plantules complètement ou suffisamment juvéniles sont produits pour servir de plantes-mères dans des pépinières commerciales pour le clonage classique. L'utilisation systématique de méthodes biochimiques de détection de la juvénilité, comme le test de la peroxydase totale, pourrait encourager les progrès dans ce domaine.

INTRODUCTION

Approximately fifteen years ago, after conducting a study on vegetative propagation of forest trees (Franclet, 1969), it was concluded that the only forest tree species which could be successfully regenerated by this means were poplar (Populus L.) in Europe and cryptomeria (Cryptomeria D. Don) in Japan. These two species were considered as the most productive.

Since that time, other species have been successfully cloned from mature trees. This may be due to improved cloning methods, to a better understanding of the maturation process, or to the possibility of reversing, if not suppressing, the effects of maturation as a result of rejuvenation and invigoration (Roberts, 1979). At AFOCEL, we have successfully cloned Prunus avium, Sequoia sempervirens, Eucalyptus spp., Pinus pinaster, and Sequoiadendron giganteum from rejuvenated mature trees.

The most outstanding results on a world-wide basis were obtained with Eucalyptus. Our first trials to clone mature Eucalyptus were in Northern Africa from 1954 to 1972. Subsequently, Martin and Quillet (1974) successfully adapted this method to the conditions of the Congo and, in less than ten years, the average yield of the plantations has increased from 7.0 to 10.0 m^3 ha^{-1} a^{-1} to 40.0 to 45.0 m^3 ha^{-1} a^{-1} as a result of clonal forestry (Delwaulle and Jean, 1981). Comparable and even better performances are presently being obtained by the Aracruz Co. in Brazil after further development of this method by Ikemori (1976). These results have been described as "a revolution in forest genetics" (Zobel, 1981).

It should be noted that twenty to thirty years elapsed between the time the method of cloning mature trees was developed and its

application on a commercial level. Prior to extending the true cloning of mature trees to all economically-important species, basic research should be conducted on the rejuvenation of meristems as recommended by Bonga (1982).

THE MAIN DIFFICULTIES ENCOUNTERED IN TRUE CLONING OF MATURE TREES

A poplar clone consists of genetically and physiologically homogenous plants, while in species which are not as easily cloned, the memorized functions gained by the buds in the various parts of the crown are more diversified and more difficult to standardize. Therefore, clones are more heterogenous when taken from older mother trees and various parts of the crown. In this case, selection within one clone is fully justified.

When cloning Sequoia, cuttings taken during the juvenile stage showed, after a relatively short rooting period (three to six months), a height growth comparable to that of the shoots of the mother tree just before severance. Although the cuttings were taken from the whole crown of the seedling, this clonewas perfectly homogenous and a selection process was not needed. Since cuttings were taken at a very early stage, the growth of this clone was comparable to that of the seedling.

Cloning during the intermediary stage, when vegetative growth was very active, required a much longer rooting period (as long as one year). However, the growth of the cutting was very different. Cuttings taken from the lower portion of the trunk (sprouts) rooted as quickly as juvenile cuttings and had rapid height growth (Ball et al., 1978). However, cuttings taken from the crown rooted more slowly and were less vigorous. In this case, selection was required. The more vigorous cuttings, despite a longer rooting period, had a sharper growth curve than seedlings of the same height. After two to three years, the cuttings were higher than the seedlings. Chaperon (1983; pers. comm.) is currently observing this with cuttings taken from five- to ten-year-old Maritime pines compared to offsprings from a seed orchard.

Cloning during the mature/senescent stage resulted in a more heterogenous clone than during the intermediary stage. However, some cuttings had exceptionally vigorous growth although the rooting period may have lasted for two to three years. The increment of these cuttings was not much lower than the better cuttings from the intermediary stage. These cuttings showed better survival, and when they are, in turn, used as mother trees, selection will be made based on vigour. In addition, they are more numerous in successive generations. This could explain the invigoration or rejuvenation which is observed after successive propagations.

The above example illustrates the difficulties encountered when cloning trees according to the age of the mother tree, namely:

(i) the heterogeneity of the quality of the cuttings taken from the crown for regeneration and vegetative vigour

(ii) reduction of the homogeneity and regeneration readiness of the organs, as well as the recovery of vegetative vigour even when the cuttings are taken from the most favourable zones and the cloning methods are more sophisticated, and

(iii) transmission and memorization of heterogeneity in the rooted cutting in successive generations.

PRESENT CONCEPTS AND THEORIES ON REJUVENATION

It has been attempted to associate the above difficulties with a number of anatomical, morphological, biochemical, or physiological criteria evolving together with the aptitude of the cuttings to regenerate organs and gain normal growth and development (Table 1).

Particular attention has been devoted to the onset of flowering. It is often considered that the onset of flowering is the end of the favorable juvenile period for gaining cuttings. However, this may be just coincidental; flowering Eucalyptus seedlings which can be readily propagated may be found in the nursery. In describing Douglas fir, Roberts (1979) wrote "it is well-established that the rooting potential of most conifer cuttings decreases with the increasing age of the tree from which the cuttings are taken. It has been assumed that this is the result of the onset of flowering, vegetative maturity, or loss of juvenility. A ten year study of Douglas fir shows that this may be the wrong conclusion. Cuttings from fifteen- to eighteen-year-old seedlings, which are now in cone production, are rooting as well as at any time in their history".

Too much dogmastism has prevailed in this field and relative value should be attributed to the criteria for the juvenile or mature stages. All parts of a tree do not mature equally. There are probably seasonal cycles where meristems are juvenile or mature.

The existence of more juvenile zones in a tree has long been recognized. Passecker (1947) showed that the part of the tree near the root remains juvenile, while the other parts are more mature (Figure 1). However, recognition of juvenile zones is often not easy. Nozeran (1978) found zones with juvenile tissue in the vicinity of flowers. A detailed examination of the leaf morphology of Eucalyptus, where up to five leaf types can be observed during the development of some species (Penfold and Willis, 1961), showed reiterations of juvenile functions at the beginning of the growth of twigs. These reiterations are more marked on sprouts after hedging, even in the crown of old trees. Comparable observations have been made by several other scientists. Assaf (1966) describes a zone in several fruit trees which is favourable to rhizogenesis at the level of tissues formed at the time of reactivation of apical meristems; the same observation was cited by Favre (1977) for vine. In Douglas fir, Strullu (1976) noted the cyclical reiteration of juvenile criteria at the beginning of growth flushes - the presence of a few amphistomatic leaves. Allen and Owen (1972) also observed the zonation of stages in the annual growth of Douglas fir.

Table 1. Juvenile and mature characteristics

	Juvenile	Mature	Source
nucleus	small, surrounded by a thin layer	reticulated and elongated surrounded by endoplasmic reticulum membranes	B
chromatin	decondensed	condensed methylated DNA polyploid euchromatin	B
ribosomes	free	associated to membranes	B
apical meristems	small dome few large cells	high dome, numerous small cells, increased RNA	B
leaves	typical form (simple or complex)	typical form (complex or simple)	B
	large epidermic cells	dense venation	B
	winter retention	thick	
	unequal opposed stomate distribution	amphistomatism	F
		frost resistant	F
		often blue coloration	F
branches	acute angle thick and long	obtuse angle thin	B
thorns	frequent	rare or none	F
bark	thick	thin	F
cuttings	easy rhizogenesis	slow rooting	
	easy recovery of vigourous growth	low and durable rate of growth	B
	quick recovery of orthotrophy when cuttings plagiotropic	low recovery of orthotropic habit	F
	quick formation of taproots	slow recovery of taproots	F
	cuttings somewhat attractive to game	cuttings very attractive to game	F
biochemical	none or weakness of ABA and other inhibitor content	high basal content in ABA and other inhibitors	F
	high content in endogenous auxins	low content in endogenous auxins	F
	high total peroxydasic activity at the basal part of excised cuttings	low total peroxydasic activity at the basal part of excised cuttings	F
	high K/Ca ratio (7)	low K/Ca ratio (1 - 2)	F
	no internal contaminant	frequent endogenous germs	F

(Source: Bonga, 1980 (B), completed by Franclet (F))

Figure 1. Juvenile, intermediary, and mature cones on a tree: the juvenile zone on the annual shoot becomes smaller as the tree grows older (Passecher, 1947).

A theory, in contrast to Passecker's (1947), was developed by Krenke (1940) after his observations of holly. It led us to propose a more complicated theory than Passecker's (1947) that juvenile territories may be found in the crown of old trees (see Figure 2). It should be pointed out that the duration of the juvenile stage of apical meristems during each flush or cyclical or polycyclical growth is shorter when taking place further from the roots. In the mature stage and in some species, this reiteration may disappear almost completely.

Passecker's theory is well-adapted to the concept of irreversibility of maturation as developed by Robinson and Wareing (1969), Olesen (1978), and Roberts (1979). According to these authors, the development criteria bound to maturation, such as loss of cloning aptitude and onset of flowering, are genetically programmed in the initials of the apical meristem. These criteria are considered to be irreversible. The species could only survive by meiosis which would eliminate the causes of senescence. Roberts (1979) tempers this irreversibility by adding the adventitious formation of buds to the possible treatments for recovering juvenility. This concept seems to imply determination at the level of the initials of the apical meristem, and, therefore, a fixed cell organization.

A second concept, reversibility of maturation, considers the relation of the meristem and the developmental stages of plants. This concept must be connected to the ideas developed by Plantefol (1951) and Buvat (1955) on the functioning of the apical meristem of the shoot - the theory of foliar helices and, more precisely, the theory of the inexistance of apical initials. It originates in cytological observations which shows that cells from the meristem are not the most juvenile and are removable in their function. Cells from the initials, which will develop into future leaf primordium, are effectively submitted to a rejuvenation induction before their development. The reaction of the cells, which are not necessarily juvenile, towards a rejuvenating impulse from underlying tissues explains the functioning of the meristem and foliar helices. Induction of juvenile or mature growth does not originate from reactivated cells, but is the result of the general state of the plant and, more

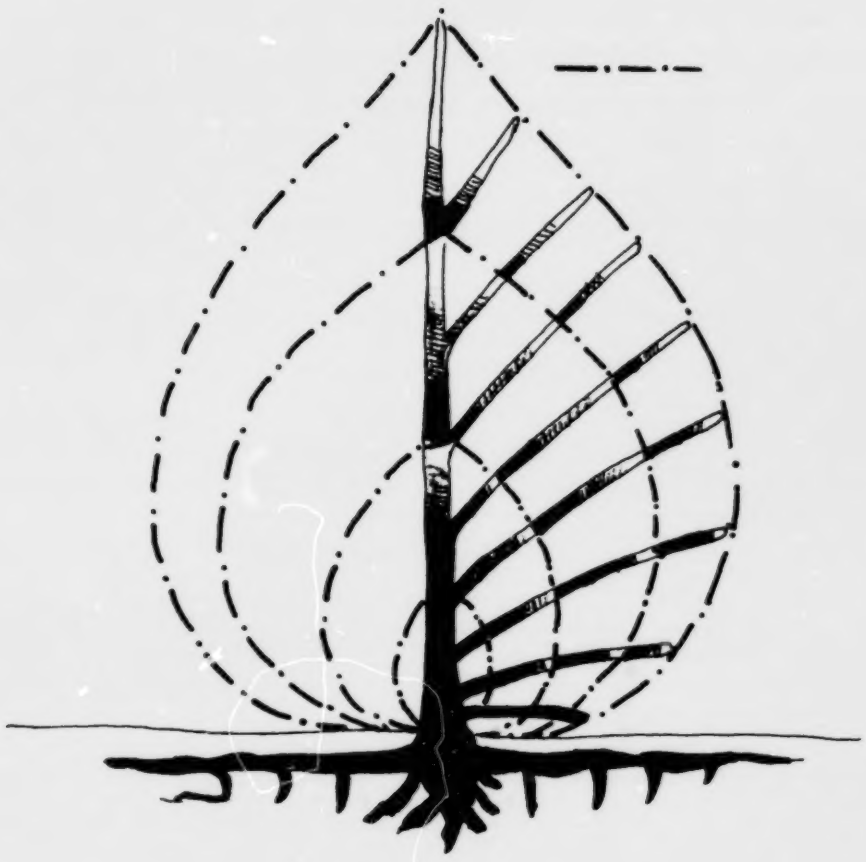


Figure 2. Juvenile, intermediary and mature zones on a tree (after Krenke, 1940).

precisly, of underlying tissues. In this case, the main role is no longer held by a cell or group of irreversible and genetically-programmed initials, but by a group of cells submitted to a correlative impulse as a result of their position at a given period.

The study of cytological oscillations in the vegetative point suggests that the meristem is maintained by an unstable equilibrium between the natural tendency of each cell to differentiate from a juvenile to a mature stage and the unknown physiological actions which are typical for plants which maintain, or create new, meristematic cells (i.e. inhibit differentiation or promote dedifferentiation) (Buvat, 1952).

This concept is strengthened as a result of recent discoveries by Erickson and Kuhn-Silk (1980) on the kinetics of growth near the apex. They show the constant existence of relative movements of cells within the tissues underlying the meristem. This movement is inconsistent with the idea of determined tissues.

With respect to the rôle of underlying tissues or the determination of the meristem, Pfirsh (1978) showed that plagiotropy of cuttings from suckers of Stachys sylvatica depends on the number of nodes in the cuttings. Very short cuttings loose the plagiotropic habit, while cuttings with more than one node still have plagiotropic growth. An accumulation of abscissic acid was observed in the first node. More recently, Paton et al. (1981), using Eucalyptus, showed the importance of the role of mature leaves for the synthesis of inhibitors for inducing or maintaining a mature meristem - a concept first demonstrated by Franks and Renner (1956) using ivy.

A third concept is that maturation would not be located in the nucleus of cells, but would be explained by the accumulation of self-reproductive determinants within, or in the vicinity of, the cytoplasm. This concept should be considered when studying maturation and rejuvenation. Certain functions during several generations of cells could be memorized. These self-reproductive determinants could be found similar to fungi, virus, or plasms, and would be at the limit of pathogenesis or symbiosis. Nevertheless, they would be more implied in senility than in maturation.

Maturation is related more to the level of organelles containing DNA in the cytoplasm or the nucleus. Consequently, genetic information would come from the organelles. This cytoplasmic DNA seems to be effectively implied in the cytoplasmic maturation of some algae or fungi, such as Acetabularia (in Bonga, 1982).

The endogenous germs, which accumulate in the tissues underlying the vegetative point, are more than likely responsible for the difficulties of micropropagation of old trees. They force us to use the cultivation of meristems (Morel et al., 1952). However, while the meristems of young trees can be easily cultivated, a number of problems occur with old trees (Jones, 1978; Margara, 1982).

These concepts confirm our theory that true vegetative replicates may be obtained from mature trees, either by using cells which remained juvenile at the base of the tree or in the meristems, by initiating ontogenetic rejuvenation, or by suppressing the determinants of maturation from meristematic tissues or cells.

REJUVENATION EXPERIMENTS IN FOREST TREES

Introduction

The first experiments on rejuvenation were probably those conducted by Doorenbos (1953) using ivy (Hedera helix). This plant has two very different forms: (i) a juvenile creeping form with adventive roots along the stem and a good aptitude to vegetative propagation, and (ii) a mature, flower-bearing form erected with a 2/5 phyllotaxy, with oval-shaped leaves, no adventive roots and is very difficult to propagate vegetatively.

Doorenbos (1953) made several grafting combinations, such as mature grafts on juvenile root stocks and juvenile grafts on mature root stocks. Mature grafts on mature root stocks were used as a control. His trials also included leafless grafts and/or root stocks. The results obtained are summarized in Table 2, where V+ is the vegetative leafy form, and G is the mature or generative form. The mark - means that leaves were removed. 0 to 40 indicate the mature or juvenile stage of the ten replications observed after a few months. 0 corresponds to the mature, flower-bearing stage, and 40 corresponds to maximum juvenility.

Table 2. Results of different grafting combinations

Root stock \ Scions		V(40)		G(0)	
		+	-	+	-
V(40)	+			10.5 / 40.0	28.2 / 40.0
	-			6.6 / 40.0	22.5
G(0)	+	40.0 / 12.2	40.0 / 6.7	0 / 0	
	-	40.0 / 40.0	40.0 / 16.3		

(from: Doorenbos, 1953)

Note: The upper figure corresponds to the juvenility of the scions
The lower figure corresponds to the juvenility of the rootstock

Grafting scion G on leafy root stock V induced stronger rejuvenation when G was leafless (28.2) rather than leafy (10.5). When the juvenile rootstock was leafless (V-), rejuvenation was weaker (6.6 and 22.5, respectively). Complete rejuvenation (40) was obtained with three leafless rootstocks grafted with a juvenile leafy scion.

Franks and Renner (1956) were successful in transmitting juvenility from juvenile to mature ivy twigs by rooting them in the same vessel filled with a nutrient solution. The concluded that a soluble element is emitted from the juvenile to the mature twigs which changes the functioning of the apical meristem. They also mentioned the rejuvenating power of frost and x-rays applied to mature twigs.

Robbins (1957) showed that spraying mature ivy plants with gibberellic acid influenced rejuvenation. A few years later, Hackett (1970) attempted to explain the reasons for the difficult rooting of mature ivy. He used *in vitro* cultivation of juvenile and mature apices

and methanol extracts from these apices. IAA or NAA, with or without the addition of catechol, were added to the medium and light intensity was controlled. He observed that in the juvenile apices, there was a strong synergy between the juvenile stage, IAA, and catechol. The few rootings obtained with mature plants were due to the combination of catechol and auxin with a long light intensity.

Miller and Goodin (1976) used the same method to study the effect foliar application of gibberellins had on rejuvenation. They rejuvenated the mature phase and demonstrated this rejuvenation by a statistical study of the in vitro propagation ratio of the callus produced. As a result of the gibberellin application, rejuvenation was obtained in less than ten weeks.

Continuing the work on ivy, Banks (1979) obtained complete ontogenetic rejuvenation with callus cultures and somatic embryogenesis. The young plantlets which were obtained did not show any intra-variability and are probably true genetic copies of the mature mother plant (Hackett, pers. comm.). Rejuvenation of ivy is a very encouraging model for the work we have initiated with forest trees.

Rejuvenation of Eucalyptus

In 1950, a french forester working in Morocco discovered the possibility of vegetative propagation of Eucalytpus. While conducting our trials in Northern Africa, it was not unusual to decrease the height of the seedlings to 25.0 to 30.0 cm to reduce evaporation prior to sending it to the planting area. During this operation, small, herbaceous shoots fell on the ground and rooted in a few days as a result of the humidity and regular sprinkling.

During the following year, trials were undertaken in two other nurseries with Eucalyptus gomphocephala, E. camaldulensis, and hybrids of E. robusta. Several thousand rooted cuttings were already planted in the forest when we initiated our work on breeding Eucalyptus. It was recognized by scientists (Franclet, 1956, 1963; Giordano, 1956; Pryor and Willing, 1963) that each Eucalyptus species could generate roots when cuttings were taken from juvenile mother trees. This was generally limited to the first fifteen nodes after the cotyledons. This is an example of rooting ability being limited to the zone near the base of the trunk and root system. It should be noted that this is no longer the case when Eucalyptus is grown under new climatic conditions. Mature E. robusta planted in Hawaii showed aerial roots up to 10.0 m from the ground (Pryor and Willing, 1963). Some species, such as E. deglupta, are easily propagated, even when using mature twigs from the crown. This confirms our reservation concerning the relative value of specific criteria of development, namely, flowering = maturity = difficult vegetative propagation.

In Morocco, where Eucalyptus grows in a dry climate, it seemed impossible to clone cuttings which were taken after the fifteenth node, or to propagate twigs from the crown of forty-year-old trees. During the

winter of 1954-55, in the forest of Lalla Ito, we selected twenty-four trees with an average height of 47.0 m and no cracks of the bole after felling. After peeling, tree #23, possibly a hybrid of E. tereticornis x camaldulensis, was considered the best for wood quality. We tried the methods known at that time for cloning. Twigs and seeds were taken from the crown after felling and sprouts were taken three months after felling for vegetative propagation and grafting. The results are summarized in Table 3. The best result was obtained six months after felling - 8.0% out of one hundred and eight cuttings.

Table 3. Vegetative propagation of sprouts taken from tree #23 - forty-years-old

Date of collecting and inserting	Number of Cuttings	% of rooted cuttings	Hormonal treatment	Miscellaneous and remarks
4.8.55	170	0	None	Herbaceous coppices three months old
11.10.55	180	8	24 hours dipping in 1/10.000 AIB sol.	Lignified coppices six months old
	65	0	24 hours dipping in 1/10.000 ANA sol.	
23.3.56	200	0	Cowdung coating	
	22	10	Talc powder with 1/1.000 AIB	
	8	0	Talc powder 1/1.000 AIB and ANA	Lignified coppices diameter size 5 - 8 mm
30.5.56	100	2	Talc powder 1/3.000 ANA	Tall coppices
19.9.56	15	7	Talc pwoder with 1/1.000 AIB	New coppices from the stump

(from: Franclet, 1956)

Table 4 shows the results of cuttings taken from the first generation of rooted cuttings. The rooting success in this case reached 65.0% out of the one hundred cuttings.

Table 4. Cuttings taken from first generation rooted cuttings

Date of collecting and inserting	Number of Cuttings	% of rooted cuttings	Hormonal treatment	Micellaneous and remarks
30.3.56	25	60	Talc powder 1/3.000 ANA	
23.5.56	75	20	Talc powder 1/3.000 AIB	
11.7.56	50	24	Talc ANA 1/3.000	Homogeneous cuttings
	50	48	Talc AIB 1/3.000	
	50	46	Talc AIA and ANA 1/1.000	
	50	12	none	Control
28.8.56	100	65	Talc ANA+AIB 1/1.000	Well-established mother plants
	100	42	Talc ANA+AIB 1/1.000	Recently-established mother plants
13.9.56	24	58	Talc ANA AIB 1/1.000	Cutting with apex (terminal)
	24	20	Talc ANA AIB 1/1.000	Subterminal cuttings

(from: Franclet, 1956)

These experiments, conducted in 1955 and 1956 (Franclet, 1956), showed that old trees which had lost their ability to propagate vegetatively could be propagated when usual nursery methods were used.

The main rules for cloning Eucalyptus were thus established in 1956. They were developed further by Franclet (1970) in Tunisia, in the Congo by Martin and Quillet (1974), and in Brazil by Ikemori (1976).

In 1956, while grafting pieces of sprouts from Lalla Ito #23 to plant in the first seed orchard, we noticed that the grafts were producing juvenile leaves. Some shoots taken from these grafts in 1956 produced rooted cuttings with juvenile leaves. Therefore, in 1962, we used these grafts to rejuvenate Eucalyptus from twigs taken from the crown.

Among the numerous experiments we conducted in Tunisia (Franclet, 1972), one was particularly interesting. We grafted twigs taken from the crown of an eighty-three-year-old Eucalytpus camaldulensis. Juvenile root stocks were raised from seeds collected from the mature tree to reduce incompatibility, and grafts taken from the old tree were grafted on these four- to six-month-old seedlings. Three treatments, characterized by the amount of time between the first and successive graftings, were applied. The first treatment consisted of only one grafting per year, and the second consisted of two graftings per year. For the third treatment, grafting was done at two to three month intervals. Only the third treatment rejuvenated the clone. Soon after the third grafting, the clones which had recovered juvenile characteristics and the growth habit of the controls raised from seeds of the same tree could be propagated.

Figure 3 illustrates the method applied in the third treatment, namely, cleft grafting for the first two graftings and plate grafting for the third. The plant on the right is a rooted cutting taken after the third grafting (Franclet, 1977).

Figure 3. From left to right: morphological changes in leaves borne by the same apical shoot in successive graftings onto juvenile root stock every second month. After the third transfer, the clones had recovered their juvenile characteristics completely (right).

Subsequently, Cauvin and Marien (1979), using this method, rejuvenated old Eucalyptus clones and produced the rooted cuttings needed by AFOCEL for its work on frost resistance.

Paton et al. (1981) attempted to explain what occurred during rejuvenation induced by grafting a mature scion onto a juvenile rootstock. They used inhibitor G - a very good biochemical factor which had previously been studied (Paton et al., 1970). Grafts from the crown of a fifteen-year-old Eucalyptus grandis were selected. The leaves from these scions had a very high level of inhibitor G (7.5 mg/g fresh weight, or more) and were impossible to root. Juvenile rootstocks were grafted above the fourth node. Between six months and two years after grafting, when diameter growth of the scions exceeded 1.0 or 2.0 cm, the scion was defoliated and the stem was strangulated to produce epicormic shoots. The treatments varied the distance between the epicormic buds and the grafting point. Juvenility was estimated according to two criteria: (i) rhizogenetic capacity; and (ii) the level of inhibitor G. The results are summarized in Table 5.

Table 5. Level of inhibitor G in the epicormic shoots and vegetative propagation readiness

Maturation criteria \ Distance from roots	Near	Far
Rooting	30 - 50%	0
G level mg/g f.w.	0.28	2.3

(from: Paton et al., 1981)

The level of inhibitor G in the epicormic shoots growing from the scion near the grafting point was comparable to that of the sprouts. However, in this case, it was a recovered juvenile characteristic and not a maintained one. Rejuvenation depended on the distance between the buds on the old scion and the root of the juvenile rootstock. Grafting and the four nodes of the root stock had no influence on the rejuvenation gradient produced by grafting.

This experiment also showed that it is necessary for the initials of future epicormic shoots to remain in the vicinity of the roots for at least six months.

This type of experimentation opens the way to new analysis perspectives on the rejuvenation phenomenon which are still largely unknown. To date, rejuvenation by grafting and stimulation of epicormic shoots as close as possible to the roots is the best method for in vitro cultivation of old clones of frost resistant Eucalyptus (Durand-Cresswell et al., 1982; Boulay, 1983).

In the field, researchers have come up against the impossibility of eliminating endogenous germs accumulated in old tissues taken from the lower part of the crown. Only meristem culture could eliminate this obstacle.

To date, we have obtained a 50.0% introduction ratio for in vitro cultivation of thirty-year-old trees as a result of rejuvenation obtained by grafting mature scions onto juvenile rootstocks and cutting back to stimulate juvenile epicormic shoots. After ten cultivations, the number of explants is reduced because of infection and lack of reactivity. The propagation ratio then increases progressively. When it is over 5.0, the strain is considered rejuvenated or reactivated. However, at this level, the clone is not yet fully free from endogenous germs. Figure 4 shows the evolution of clone #16, taken from a thirty-year-old tree, after a three to four week cultivation period. Figure 5 illustrates rejuvenation obtained after thirteen cultivations. After the clone has been rejuvenated, propagation and in vitro cultivation are as easy with an older clone as with a juvenile clone. Nutrient, vitamin, and hormone requirements are simplified. The whole production cycle could be planned on the KNOP medium without growth substances added. Once the problem of contaminants has been solved, probably through cultivation of rejuvenated meristems, this will likely be the best and safest method to multiply old, selected genotypes by somatic embryogenesis. However, we feel that the problem of rejuvenation should be solved first before tackling this attractive step (from the point of view of lowering production cost).

The relatively expensive cost of in vitro cultivation by axillary budding is essentially due to the cost of labor. These costs could be amortized over a sufficient number of plants (vitroplants) delivered at the rate of 500 per clone and used as initial mother trees in industrial nurseries. Once production has started, each nursery can become self-sufficient by taking cuttings from young, rooted cuttings from the preceding generation.

There are two problems which currently justify in vitro cultivation: (i) to improve rejuvenation of clones; and (ii) to rapidly produce a sufficient quantity of rejuvenated cuttings to enable nurseries to become self-sufficient.

Chaperon (1983) collected ten cuttings each month from each mother tree raised in vitro. Two or three months after the season, these cuttings will, in turn, yield ten cuttings before being used in plantations. With such a multiplication rate and easy rootability, it is not practical to produce in vitro plants to be used for reforestation.

Other Broadleaved Species

Eucalyptus sprout easily, as do most hardwood species, and the juvenile stage may be easily maintained. Several other hardwood species, such as aspen and hybrid aspen, have been successfully rejuvenated not only at our Institute, but in other laboratories as well.

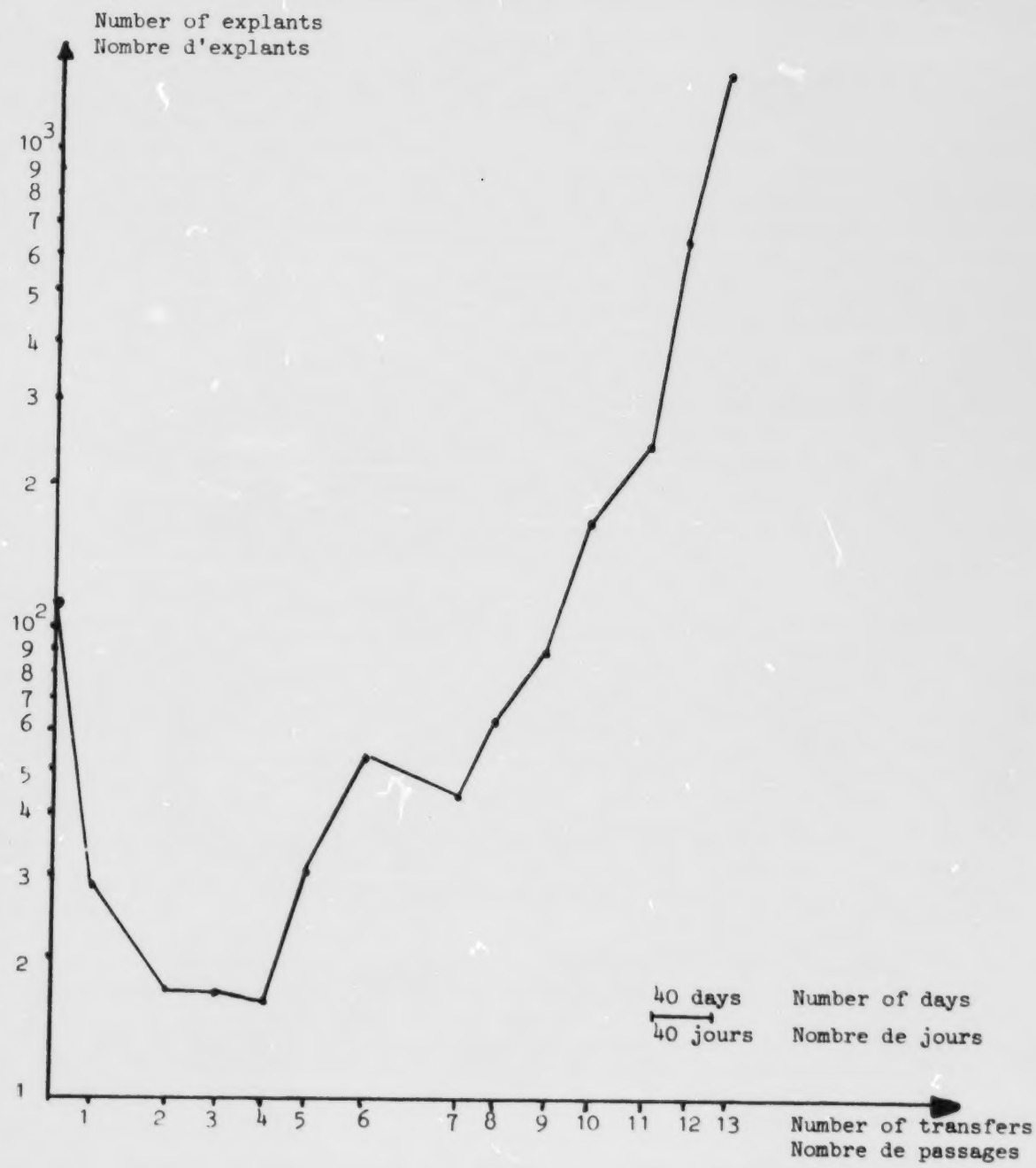


Figure 4. Evolution of clone #16, taken from a thirty-year-old Eucalyptus, after a three to four week cultivation period.

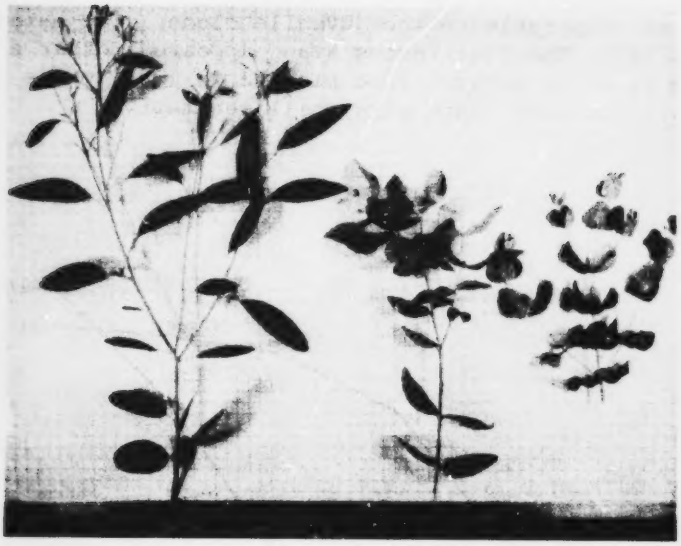


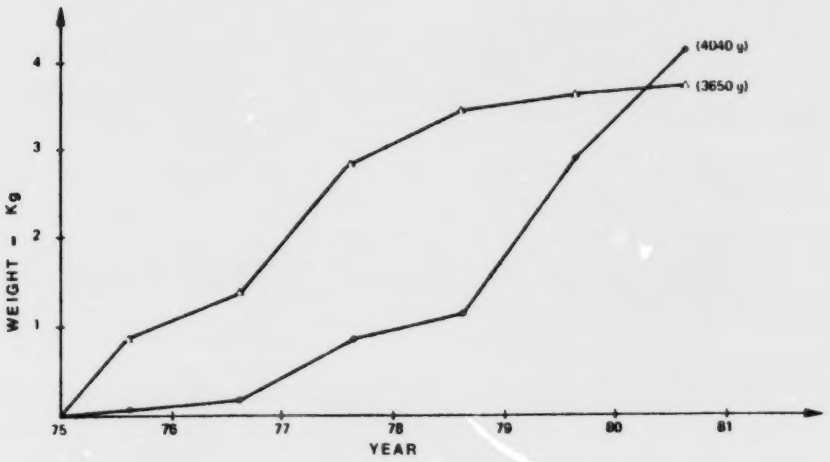
Figure 5. Rejuvenation of clone #16 obtained after thirteen cultivations. From left to right - scion from the crown of the ortet, scion from the third grafting, and twig from plant obtained after thirteen cultivations in vitro

Using pieces of roots, it is easy to grow herbaceous buds (suckers) which are, at some point, morphologically juvenile. These herbaceous shoots are rooted under mist. Juvenile clones can then be produced by taking cuttings from the rooted shoots. Nodes from these plants can also be used for in vitro propagation. Clones produced from mature trees using this method are grown for several years and are very homogeneous. Destremau and Franclet (1982) used this method for cherry trees which resulted in the commercial production of clones issued from mature trees which had been rejuvenated and propagated in vitro.

Comparable experiments are presently being conducted with walnut; more precisely Juglans vilmoriniana which is the oldest described hybrid of J. nigra x regia. This tree, planted in 1816 at Verriere le Buisson, is presently 4.06 m in girth. We succeeded in grafting it onto young walnut trees and the rejuvenation process in now underway.

For Platanus acerifolia, we used the collection at Kew Garden which was planted in the seventeenth century, and the tree planted in 1785 in the Paris botanical garden. Flower-bearing shoots were taken from the crown and rooted in 1973. At the same time, seeds were sown to obtain a juvenile control clone.

The mature and juvenile clone were planted in 1975. Each year, the shoots from the preceding year were cut back. Figure 6 illustrates the evolution of the average weight (about ten trees per clone). Using the method of cutting back, seven years elapsed before the yield of the mature clone was comparable to the juvenile clone. The mature clone bore flowers until 1977. The floriferous stage appeared rather early on the cuttings taken from the mature clone planted in 1980, while cuttings taken from the juvenile control clone were still vegetative.



o mature clone
Δ juvenile clone

Figure 6. Evolution of the average weight of coppices of Platanus acerifolia from stumps of juvenile and adult stock plant after annual cutting back

Repeated cutting back favours rejuvenation which is practical for true cloning of old trees. However, rejuvenation takes place slowly and is probably incomplete. A forester may consider it sufficient for the purpose of reforestation, but the physiologist would like to improve it. This type of incomplete rejuvenation justifies the word "invigoration", preferred by Roberts (1979), rather than "rejuvenation".

In fruit trees, a complete ontogenetic rejuvenation was obtained by Jones (1978) by apex culture. While it is more difficult to obtain a successful meristem cultivation with old trees, once it has been obtained, plantlets from an eight-year-old tree do not differ from one-year-old seedlings.

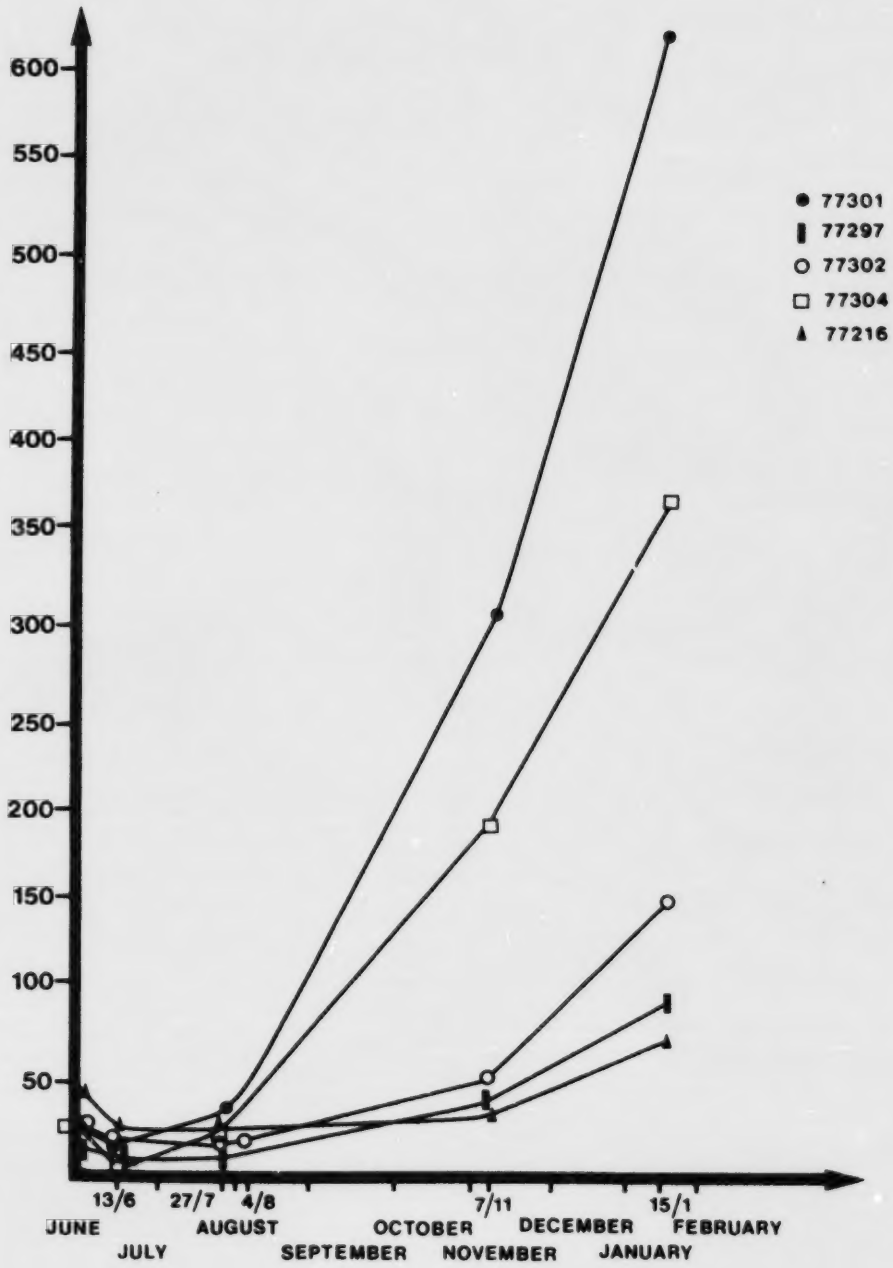


Figure 7. Sequoia rejuvenation at Anjou. Evolution of a number of buds available in vitro as a function of subcultures (after Boulay, 1978).

Experiments on Rejuvenation of Conifers

Sequoia sempervirens

Ball (1950) was the first to successfully reproduce tigelles from old trees in vitro; however, viable plants were not maintained. Murashige (1977) described his results in producing such plants from basal shoots from old trees. Boulay (1978), using this method, was the first to successfully produce a significant quantity of plants, propagated in vitro, from basal sprouts of over two hundred trees selected in France for their frost resistance. Within two years, he was able to produce approximately forty thousand rooted trees.

During the numerous cultivations needed to produce at least two hundred copies for planting mother trees, we observed that a type of reactivation of each of our clones occurred progressively (see Figure 7). In the nursery, we noted that the first plants produced by the first cultivations had low rootability and low recovery of height growth and orthotropy. The last plants produced in each clone (i.e. the eighth and twelfth cultivation) had recovered their vigour and orthotropic growth. (Franclet, 1981).

The study of this evolution was then carried out by de la Goublaye de Nantois (1981). This scientist studied a ninety-year-old Sequoia growing in Fontainebleau and observed that the main obstacle hindering in vitro cloning of this tree was the disinfection of the explants. Those from the base of the tree were always more difficult to disinfect than those from the crown. However, basal shoots, once disinfected, were always more reactive. It was also observed that the shoots from the top recovered their reactivity and still remained relatively free of endogenous germs. Once grafted onto a young seedling, all trees were very easy to cultivate in vitro. Plants raised in vitro showed improved reactivity (vigour and orthotropy) (Table 6).

This effect was maintained, and after a one-year cultivation period in the field, the influence of the number of subcultures for a Sequoia sempervirens clone on vigour, orthotropy, and vegetative propagation were determined (Table 7).

After cutting back in November, 1981 to weigh the aerial biomass, the difference between the second and fourth cycle seemed to be insignificant. Nevertheless, there was an important difference in the vigour of the seedlings and clones.

Concurrent with this work, trials were conducted with clones selected from the forest of Arcata which were several centuries old (Franclet, 1981). Cuttings were taken from one tree in particular, S5, from three positions: (i) upper crown (radial) (Cl. 78466); (ii) lower crown (vegetative) (Cl. 78471); and (iii) basal shoot (radial) (Cl. 78469 ("topoclones").

Table 6. Evolution of the rhizogenetic aptitude within a ninety-year-old Sequoia clone according to the number of in vitro cultivations

Sequoia clone FTBr (BS and B)	Initial explants taken at the base of the tree		
	Usual in situ vegetative propagation	Vegetative propagation after two in vitro cycles[1]	Vegetative propagation after four in vitro cycles
Mean rooting time[2]	8.0	3.0	2.0
Plagiotropy notation after six months cultivation in the nursery[3]	4.8	3.5	2.7

[1] one cycle = four week cultivation on MS + BAP medium and one month cultivation on MS + activated charcoal medium

[2] the mean rooting time for one cutting lot corresponds to 50.0% rooted cuttings

[3] from de la Goublaye de Nantois et al. (1979) 5 = plagiotropy
0 = orthotropy

(from: de la Goublaye de Nantois, 1981)

Table 7. Influence of the number of subcultures for an old Sequoia sempervirens clone on vigour, orthotropy, and vegetative propagation after a one year cultivation period in the field

Number of in vitro cycles	Average weight of aerial biomass (grams)	Average number of orthotropic shoots	Vegetative propagation readiness (% of well-rooted cuttings)
0 cycle cuttings from sprouts	255.1	0.15	32.1
2 in vitro cycles of sprout pieces	290.4	5.40	44.6
4 in vitro cycles of sprout pieces	788.3	13.20	50.3
control seedlings	277.8	1.10	75.1

(from: Franclet, 1981)

As a result of previous grafting onto juvenile seedlings, *in vitro* cultivation was easier. After one cycle of *in vitro* culture, the average elongation in tubes varied from 10.1 to 16.2 mm in one month; shoots from the upper crown elongated less than basal shoots. After seven months and seven cultivations, this difference disappeared. The height of the plants grown in the greenhouse and ready for outplanting are shown in Table 8.

Table 8. Elongation after the first *in vitro* subculture, number of plants produced, and average height of the topoclone after seven subcultures and seven months in the greenhouse

Clone	Topoclone	July, 1979 Elongation after first subculture (mm)	Number of plants produced (after seven months)	July, 1981 Average height (mm)
S5	UCR[1]	10.1 ± 6.7	197	37.0 ± 9.0
	LCV[2]	14.2 ± 7.3	164	32.0 ± 12.0
	BSR[3]	16.2 ± 9.1	155	35.0 ± 8.0

[1] upper crown (radial)
[2] lower crown (vegetative)
[3] basal shoot (radial)

(from: Franclet, 1981)

These topoclones were then interplanted with younger, French clones. Presently, the three topoclones cannot be distinguished from one another. However, they are easily distinguished from the two younger French clones by the grey colouration of the leaves and the morphology of the young shoots (Figure 8).

The *in vitro* culture method which was applied suppressed the intraclonal variability which impeded cloning, but did not suppress all of the effects of maturation accumulated over several centuries. *In vitro* cloning of old *Sequoia* was confronted with the impossibility of rooting tigelles produced and elongated *in vitro*, even when their reactivity and elongation improved with successive subcultures. Rooting was still rare and sporadic (Boulay, 1978).

In vitro rooting is a maturation factor in *Sequoia*. For this research, Bekkaoui (1983) used two clones which were cultivated *in vitro* in our laboratory. They were submitted to a number of cultivations using the methods described by Boulay (1978).

Clone #77304 originated from sprouts taken from a fifty-year-old mother tree. Clone #78461 originated from ARC 154 which is said to be the tallest in the world (113.16 m) and is estimated to be five to eight centuries old (Libby, pers. comm.). Cuttings were given to us in July,

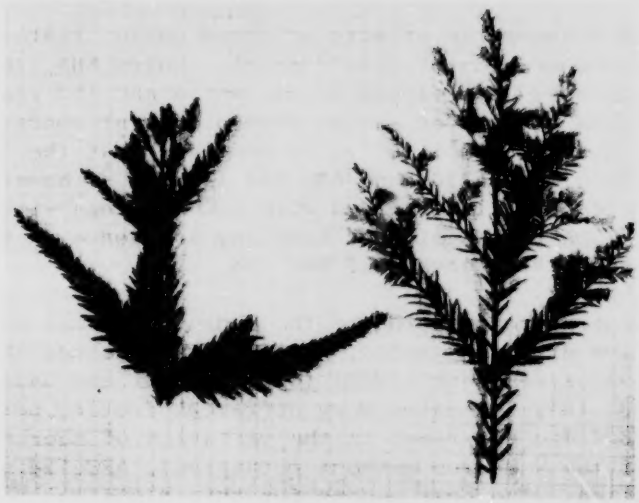


Figure 8. Foliar morphology permits the rapid distinction between mature (left) and younger clones (right).

1978 and grafted onto young seedlings. Bekkaoui (1978) used two clones from this tree:

(i) the first clone was propagated in vitro in 1978/79 (see Franclet, 1979) and has been cultivated in vitro since that time, with the period of cultivation occasionally extending to two or three months

(ii) the second clone originated from cuttings taken from the cuttings grafted in 1978. It was introduced in vitro in December, 1982 and submitted to less transfers than the first clone

Boulay (1978) showed that clone #77304 propagated and elongated in vitro as easily as a juvenile clone, but only rooted occasionally. De la Goublaye de Nantois (1981) showed that clone #78461 could not be rooted in vitro using the usual methods. Shoots which are propagated and elongated in vitro are more easily rooted in situ (Poissonier et al., 1981).

In his current work, Bekkaoui (1983) has been successful in rooting in vitro, provided rhizogenesis is first induced by an auxin treatment for a short period (one week cultivation), followed by

cultivation on an expression medium which has the same composition as the induction medium except for the auxin. He then improved the rooting percentage by optimizing some of the factors known to influence in vitro rhizogenesis.

Figure 9 shows the effects of three auxin treatments (IAA, IBA and ANA) at a concentration of 5.10^{-5} mol/1. Using ANA, the rooting percentage of clone #77304 reached 80.0%, while ARC 154 reached 40.0%. Figure 10 shows the effects of ANA at five different concentrations: 0, 5.10^{-7}, 5.10^{-6}, 5.10^{-5}, and 5.10^{-4}. It confirms that the optimum concentration for clone #77304 and ARC 154 is 5.10^{-5}; however, the optimum level must be more strictly observed with ARC 154 than with clone #77304. The addition of TIBA to ANA did not have any influence on clone #77304, while it suppressed rhizogenesis of ARC 154.

The mineral composition of the medium also has an influence. A comparison of five different media, namely Murashige and Skoog (1962) (MS); Knop (1865) (K); Nemeth (1980) (N); Tripathi and Saussy (1980) (T); and Heller (1953) (H), indicates very different rooting percentages. While clone #77304 did not react to the variation of macronutrients (with the exception of the T medium without potassium), ARC 154 showed an important variation. The best medium for this clone was N which produced a rooting percentage of 50.0%, while MS medium produced 0% rooting percentage. Special trials conducted with MS medium showed that by diluting the medium, the rooting performance of clone #77304 greatly improved, while the rooting performance of ARC 154 did not improve at all. The results obtained are shown in Table 9 and Figure 11.

Table 9. Influence on in vitro rhizogenesis of clone #77304 and ARC 154 as a result of diluting the macro-and micronutrients in MS medium

Macronutrient dilution of the base medium	Micronutrient dilution of the base medium	In vitro rooting percentage	
		50-year-old clone (Cl. 77304)	500-year-old clone (ARC 154)
full	1/3	72.0%	0%
1/3	full	75.0%	65.0%
1/3	1/3	80.0%	70.0%
1/9	1/9	75.0%	50.0%

(from: Bekkaoui, 1983)

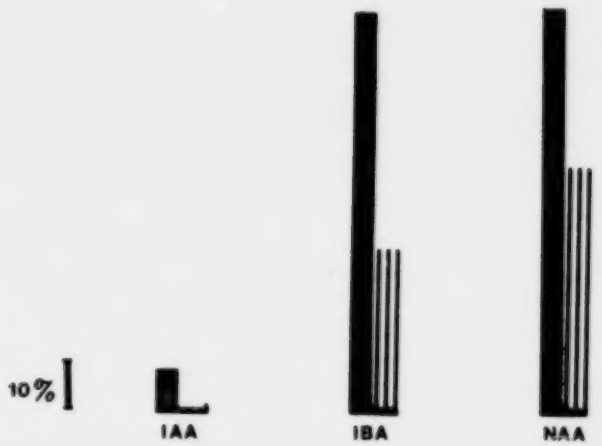


Figure 9. Percentages of rooting on two Sequoia clones of very different ages, ■ : 50 years, ▥ : approximately 600 years, after treating with three auxins at a concentration of 5×10^{-5} mol ℓ^{-1} (Bekkaoui, 1983).

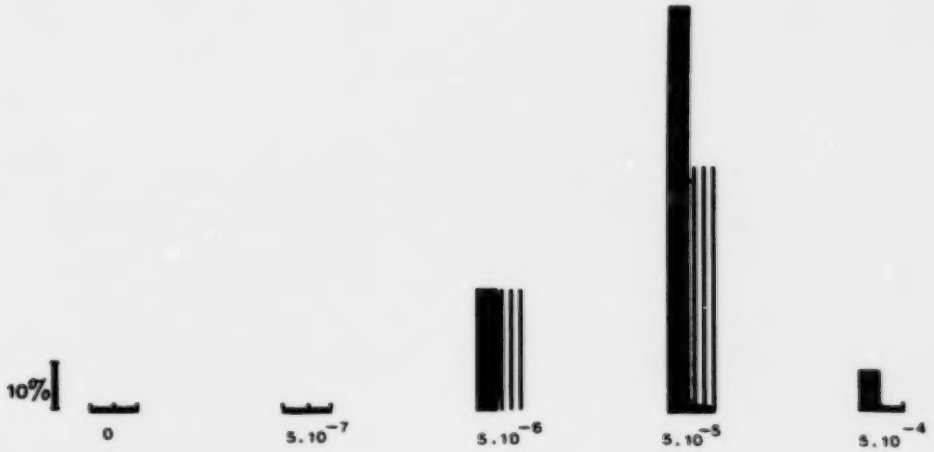


Figure 10. Percentages of rooting on two Sequoia clones of very different ages, ■ 50 years, ▥ : approximately 600 years, after treatment with five different concentrations of ANA (Bekkaoui, 1983).

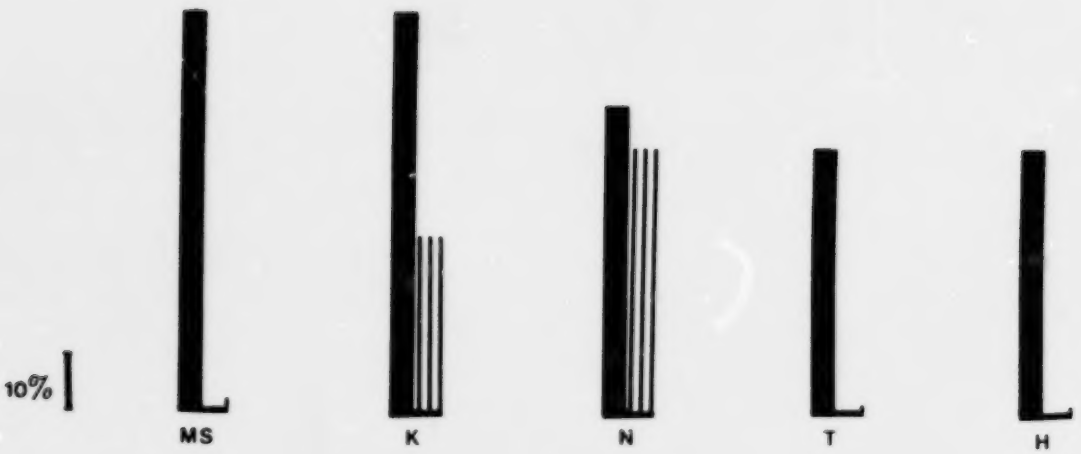


Figure 11. Influence of mineral composition of the rooting medium on the percentage of rooting of two Sequoia at very different ages, ■ : 50 years, ▥ : approximately 600 years. The treatments are: MS for Murashige and Skoog (1962); K for Knop (1865); N for Nemeth (1980); T for Tripathi and Saussy (1980), and H for Heller (1953).

The medium content in sugar and agar, and the influence of activated charcoal were also studied. I will only deal with the trials on the variation of the photo- and thermoperiod which were conducted at the phytotron of Gif sur Yvette. The following criteria were applied:

(i) night (temperature: + 22°C)
(ii) 9 hours day (40 W/m2) + 15 hours night
(iii) 9 hours day (40 W/m2) + 15 hours glowing red (660 nm-12 to 15 W/m2)
(iv) 16 hours day (100 W/m2) + 8 hours night

Except for the first treatment (full night), which reduced rhizogenesis from 75.0 to 80.0% to 40.0%, these various treatments had little influence on the fifty-year-old clone. They did, however, have a strong influence on the five hundred-year-old clone. The third treatment increased the rhizogenesis of this clone from 25.0% to 60.0%.

The comparison of various thermoperiods (5°, 12°, 17°, 22°, and 27 to 32° - nine hour day in each case) is also very interesting. Treatments 5°, 12° and 17° lasted for six weeks followed by an additional six weeks at 20 to 25° and eight hour day before being evaluated. In this instance, the fifty-year-old clone rooted more easily in each treatment than the five hundred-year-old clone. Rhizogenesis was totally inhibited by 5°, 12°, and 27° to 32° treatments. Tubes from 5°, 12°, and 17° treatments, placed at 20 to 25°, regained rhizogenesis. A six week period at 17°, followed by an additional six weeks at 20 to 25°, was good for the rooting of both clones.

This study was based on only the two clones which, therefore, makes it difficult to draw any conclusions. Nevertheless, it showed that in vitro rooting of very old clones, which was considered impossible even five years ago, is now possible. The specificity of older clones towards the criteria studied presents a number of problems concerning the strategy of future work in this field.

Using in vitro rhizogenesis as a rejuvenation criteria is not very practical because of the length of time (six weeks) required before the effect of a treatment can be evaluated. Therefore, we are attempting to correlate rhizogenesis of tigelles produced in vitro to the other criteria expressed earlier.

By cultivating the leaves of Sequoia as described by Ball (1978), Bekkaoui (unpubl. data) is attempting to develop an in vitro rooting test which would be less destructive to implants and would separate the evolution of the cultivation of tigelles from the test itself. The first results of this test are very promising. We feel that such a test will be very useful when dealing with inhibitors implied in the self-maintaining functions.

Verschoore with Tripathi and Franclet (unpubl. data) are developing the work of de la Goublaye de Nantois (1981), as well as studying the influence of grafting three topoclones from an eighty-year-old tree:

(i) topoclone #36 - from a root sucker, 50.0 cm in height, growing at a distance of 3.0 m from the stump
(ii) topoclone #37 - taken from the lower part of the crown
(iii) topoclone #40 - taken from the middle of the crown, at a height of 15.0 m

The foliar morphology of these topoclones is very different. Topoclone #36 had juvenile-like leaves, while topoclone #37 had long leaves and topoclone #40 had very short leaves similar to those of a young giant Sequoia. These three topoclones were grafted onto young seedlings and have been vegetatively propagated since December, 1981. One year later, these grafts, which were very intensively cultivated, showed very vigorous growth. In the greenhouse, they were, on average, 1.35 m in height and became orthotropic as a result of staking. Rooted cuttings, cultivated under similar conditions, showed poor growth and were plagiotropic. The foliar morphology of the grafts and cuttings, as well as the flowering capacity, were maintained.

We studied the in vitro cultivation readiness of shoots and meristems severed at different periods and attempted to correlate them to possible variation in the level of potassium and calcium in the buds during the same period. The aim of this investigation was to find a better way than that studied by Bekkaoui (1983) to estimate rejuvenation.

For the shoots introduced in the usual way in different seasons, we noted the percentage of infection by endogenous germs and the aptitude to recover growth by auxillary budding and elongation. For the meristems, we observed the aptitude to survive and grow on a medium which was specially developed by Tripathi and Saussy (1980). In the shoot cultures, topoclone #40 had less endogenous germs than topoclone #36. Infection was more important in the winter, and beginning in March, explants from every topoclone produced reactive cultures. The results of the seasonal variation of the aptitude to cultivate meristems and the K/Ca ratio in the buds of the three topoclones at three different height levels are summarized in Table 10.

After one year cultivation, important differences appeared in the relative K and Ca levels according to the topoclones. In April and June, topoclone #36, in each position, had more potassium than topoclone #37 which, in turn, was better than topoclone #40. A general increase of the potassium level in the buds was observed between April and June, and the reactivity of the meristems improved during the same period. The distance of the bud from the grafting point influenced their aptitude to produce active meristems and also their calcium level. The buds closest to the grafting point contained more calcium.

The same measures on in vitro material related to the number of cultivations are less convincing. However, the influence of the disinfection treatment, using calcium hypochlorite, on the calcium level of explants should be taken into consideration (Reynoird, 1983).

Table 10. Seasonal variation of the aptitude to cultivate meristems, and the K/Ca ratio in the buds of three topoclones at three different height levels

		Topoclones					
		36		37		40	
Period	Position on the graft	activated meristem	K/Ca	activated meristem	K/Ca	activated meristem	K/Ca
April	1 top	0/12	4.75	0/12	3.73	0/12	2.13
	2 middle	0/12	5.00	0/12	2.88	0/12	2.00
	3 lower	4/12	4.20	9/12	1.24	12/12	1.39
June	1 top	0/12	8.27	4/12	5.00	2/12	3.09
	2 middle	0/12	8.30	5/12	4.90	2/12	3.04
	3 lower	4/12	5.72	10/12	4.60	5/12	4.41

(from: Vershoore, unpubl. data)

Utilization of a characteristic such as "peroxydase activity", used by Moncousin (1982) with artichoke, would probably be better correlated with the juvenile or rejuvenated stage than with the criteria mentioned above.

With respect to the "meristem" test, it should be noted that it was the first time that plantlets from meristems of an eighty-year-old Sequoia could be observed. The plantlets were more juvenile than those obtained from cultures of shoots from topoclone #36 and it must still be verified if the resulting plants are as juvenile as the seedlings.

Meristems, after recovering active growth, were in excellent sanitary condition and free of endogenous germs. This is probably a result of grafting, as the buds near the grafting point produce meristems which are the easiest to cultivate in vitro. Excellent homogeneity of the growth and morphology of meristems from various topoclones can be observed.

Rejuvenation in Other Softwoods

The species Sequoia marks the limit between species which are easy and difficult to rejuvenate. Therefore, it was used to develop a method which could be applied to species which are difficult to rejuvenate, such as Douglas fir, Maritime pine and spruce.

Douglas fir

We have attempted to reproduce the results of de la Goublaye de Nantois (1980) obtained with Douglas fir by repeated micrografting of old

clones onto four- to six-month-old seedlings cultivated under continued light. Results obtained in 1981 are summarized in Figure 12. Four to six successive graftings, or almost two years, were needed to rejuvenate an apical bud from an old Douglas fir. Reynoird (1983), working in our lab, attempted to reactivate the buds from the same clones as those used by de la Goublaye de Nantois (1980) more rapidly by applying five to six sprayings weekly of BAP and/or GA_3. Application of BAP produced sufficient reactivation of the buds to introduce and multiply in vitro. This was also done by de la Goublaye de Nantois (1980) after five to six successive graftings. This is an important result which we are presently applying to meristem culture.

Maritime pine

Some years ago, we rejuvenated a Maritime pine clone selected at fourteen years of age from a seed orchard (Franclet et al., 1980). This clone, as well as other clones, were propagated and planted in clonal tests in Bordeaux. We are presently observing that these clones, which were initially poorer than seedlings, are now larger. This fact will favour the promotion of mass propagation of clones from trees selected in progeny trials which are between ten and fifteen-years-old. Present developmental work is bearing on the propagation of seedlings from the best families. We have carried on the study of the rejuvenation of clone #107 - a tree which is over eighty-years-old. We were successful in reinvigorating the clone after stimulation of brachyblasts by BAP, and buds were obtained which could be grafted. During six successive graftings over the past six years, the length of the needles, or pseudophylla, decreased from 46.5 cm to 12.0 cm. We are now able to graft every six months. There has been a very clear reactivation which will be used at the meristem level by applying the methods developed by Tran Van and David (unpubl. data).

Picea abies

At AFOCEL, we have developed a cloning program for Norway spruce by applying the method developed by Kleinschmit et al. (1973). We have also shown that repeated hedging of eighteen-year-old Norway spruce produced clones provided that the problem of herbaceous vegetative propagation, conducted in the summer, was overcome. At that time, we did not have sufficient control over the temperature of our greenhouse in the summer. This problem has been overcome by the use of wet tents.

Tsogas (1983) showed that it is possible to reactivate buds from twenty-year-old Norway spruce and to cultivate them in vitro. We should now try to cultivate their meristems (Figures 13, 14 and 15).

DISCUSSION

The average aptitude of ordinary shoots, taken from the crown of old trees, to grow into whole plants diminishes with age and disappears completely despite the sophisticated methods used. However, horticulturists have discovered methods to overcome this difficultly, such as natural methods, such as the use of suckers, and natural or induced

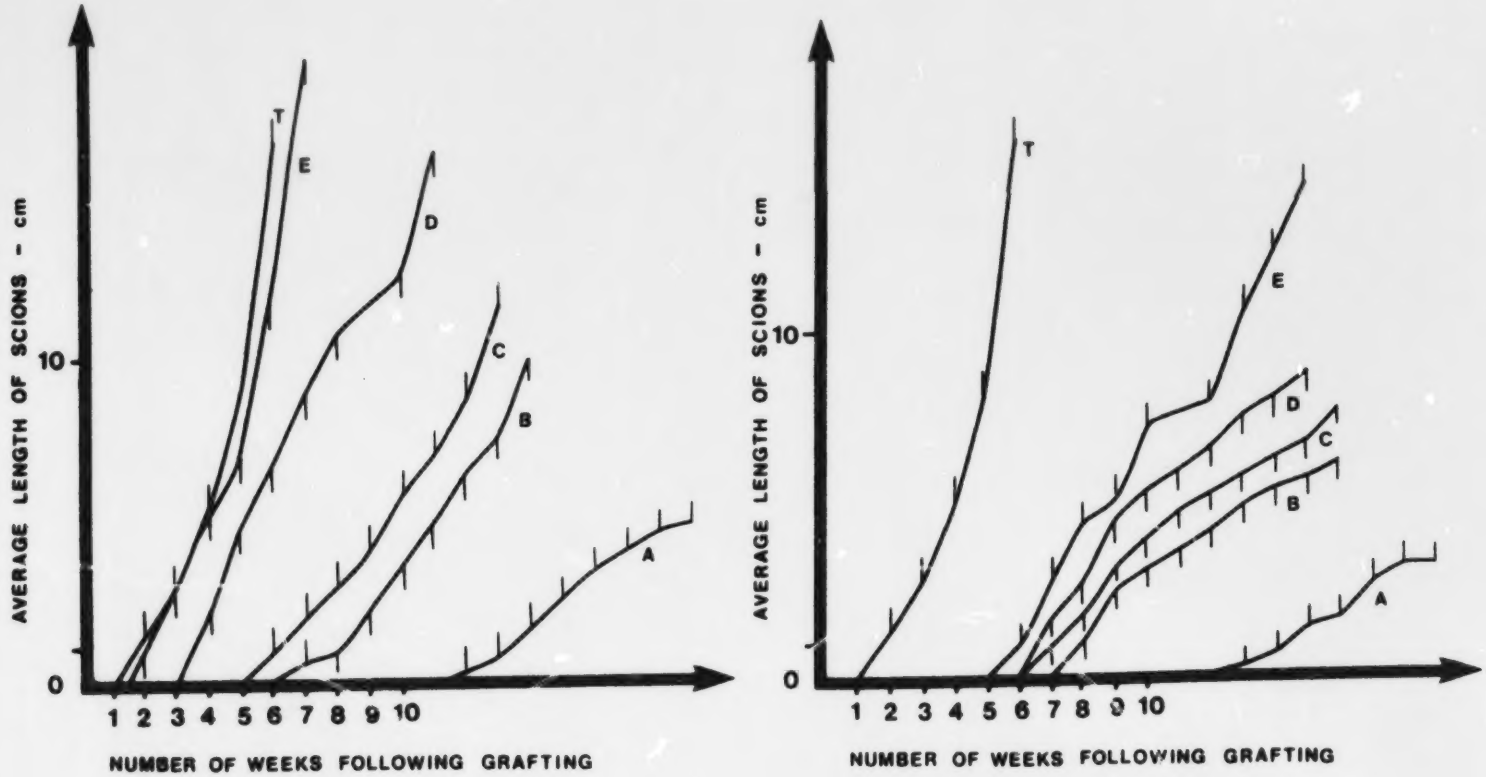


Figure 12. Progressive change during a 2-year rejuvenation period of mature Douglas fir buds successively grafted onto juvenile root stocks cultivated in 24 hour light, at 25°C. Clone "DEU 11", 95 years old (left); clone "SOR 5", 75 years old (right). (Goublay de Nautois T., 1980). The curves represent successive transfers: A, B, C, D, E,; 1st to 5th: T; control, self graft of an 8-month-old seedling. Vertical bars represent standard errors of the means at p = 5% (T test).

Figure 13. Photograph of a dormant bud on a 20-year-old clone after spraying four times in one month with BAP (100 $mg \cdot \ell^{-7}$). One may observe a slight elongation of the bud axils and formation of buds (BRe) and bud scales (Tsogas, 1983).

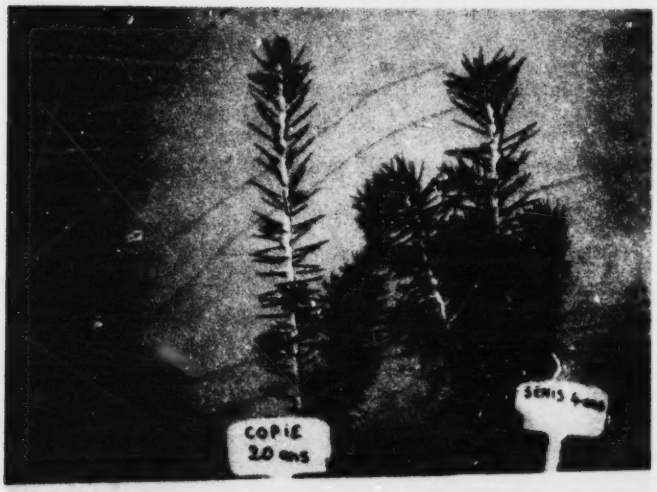


Figure 14. Flushing of vigorous, orthotropic shoots following development of adventitious buds six months after termination of treatment with cytokinins. A vigour comparable to that of shoots flushed on 4- to 20-year-old trees is observed (Tsogas, 1983).

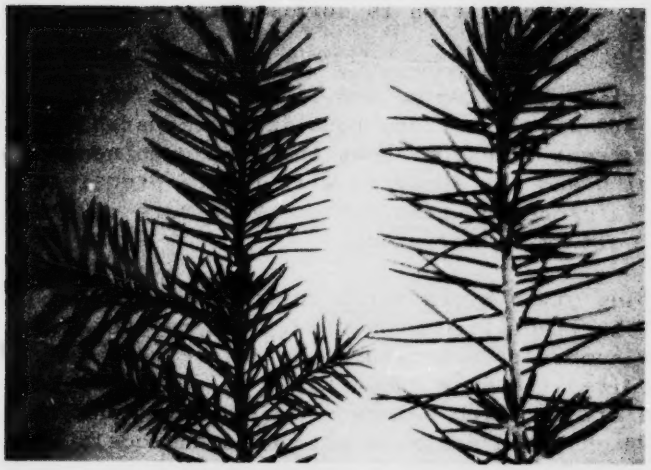


Figure 15. Comparison of current year's shoot (left) with a shoot flushed following treatment with cytokinin (right) on a 20-year-old clone. The "rejuvenating" effect of treatment with cytokinin is observed on the needle length and on the space between modes of the induced shoot.

epicormic shoots and sprouts. These shoots are capable of producing a few rooted cuttings. Cuttings taken from the original cuttings improve the rooting percentage provided that cultivation methods are correctly defined. The older the tree, the more important it is to strictly respect the defined cultivation methods.

Using rather simple methods, it is possible to clone wild cherry trees, aspen, sycamore, sequoia as well as eucalypts and walnut even though the twigs of these species are generally considered impossible to root.

Relatively juvenile clones can be obtained after a number of successive vegetative propagations. Cutting back or hedging is not always considered as true rejuvenation, but rather as selection of meristems which remained juvenile and stabilized at a maturation level compatible with an economical cloning. It is difficult to determine the reality of rejuvenation as long as it is impossible to isolate meristems to estimate their maturation level by observing issued plants.

For trees which do not produce suckers and trees without epicormic shoots or sprouts, grafting is often the only practical method to clone old trees. This method is especially uneconomical for cloning forest trees, but often favours a real rejuvenation process as indicated by the results of Paton et al. (1981). The reiteration of grafting in

appropriate culture conditions gives an old meristem the characteristics of a meristem from a seedling. Miniaturization of scions could be an important factor in the recover of juvenility. However, it should be stressed that if this condition is sufficient, it is not yet known if it is needed.

This time-consuming method is, to date, the most efficient one used to rejuvenate very old trees to a level compatible with in vitro cultivation. Current research shows that the combined effects of repeated graftings, hedging and BAP spraying will reduce preparation time before introduction of a clone in vitro either by culture of stem pieces or, better yet, by culture of meristems.

Examples given by in vitro cultivation of stem pieces show that this method is, in itself, rejuvenating. The rejuvenating effect is a function of the number of subcultures on determined media. The nature of the factors involved in these subcultures is not fully known. Is it a dilution of inhibitors which saturate the aged implant and dissolve in the culture media which often contains activated charcoal; is it an activation promoted by the prolongated influence of enriched media which contains hormones and, more particularly, cytokinines; or is it the trophic effect of minerals contained in the media? An important method of investigation has recently been discovered as a result of the use of biochemical markers, such as peroxydaze (Moncousin, 1982) or inhibitor G (Paton et al., 1981).

It is clear that in vitro culture of parts of old trees, which have been rejuvenated using the above-mentioned methods, is a powerful method of rejuvenating a clone and enables the cultivation of meristems from old trees.

Cultivation of meristems should, theoretically, free the clone of the correlative effects. This miniaturization of propagules also frees the clones from contaminating germs. We know that the cultivation of vine meristems produces rejuvenated clones from very old vine plants. It is, therefore, probable that treatments which are insufficient to induce complete juvenility in a clone will be sufficient to succeed in the culture of meristems. Therefore, after having attempted to rejuvenate old trees for indefinite in vitro cultivation, we are now engaged in the production of rooted plantlets from meristem culture.

CONCLUSION

We have often obtained rejuvenation for a number of trees without knowing why and what due to insufficient knowledge. Our present methods are not being adapted, are too time-consuming, or are not economically feasible.

The results obtained with Eucalyptus, Sequoia, and Maritime pine were very promising and promoted further research. We will, therefore, obtain a large quantity of clones in two very different ontogenetical stages. This plant material could then undergo various biological

analysis to try to explain the problems presented in this paper. It is likely that the explantion of these phenomena will lead to a complete modification of our present cultivation methods.

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SESSION III

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VEGATATIVE PROPAGATION IN EUCALYPTUS

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ABSTRACT

Aracruz Florestal uses rooted cuttings almost exclusively for the 12,500,000 trees it plants annually. The reasons the company concentrates on rooted cuttings are briefly outlined in this paper. Growth improvement has been dramatic, increasing from 36 m^3 ha^{-1} yr^{-1} for the original unimproved "Brazil source" Eucalyptus grandis to 64 m^2 ha^{-1} yr^{-1} on a six-year rotation. The clones used are essentially free from canker disease.

The methodology used at Aracruz to obtain and treat cuttings for rooting is outlined in the paper. Aracruz has been able to mechanize the system, and the cost of producing rooted cuttings is similar to the cost of growing seedlings.

RESUME

La compagnie Aracruz Florestal plante chaque année 12,500,000 arbres et utilise à cette fin presque exclusivement des boutures racinées. Les raisons de sa préférence pour les boutures racinées sont exposées brièvement. Les gains ont été considérables, la production étant passée de 36 m^3 ha^{-1} an^{-1} pour la source brésilienne d'origine, non améliorée, d'Eucalyptus grandis à 64 m^3 ha^{-1} an^{-1}, avec une révolution de six ans. Les clones employés sont essentiellement indemnes de maladies à chancres.

La méthode employée par Aracruz pour obtenir et traiter les boutures à raciner est décrite. La compagnie a pu mécaniser ses opérations, et son coût de production est similarie à celui de la production de semis.

INTRODUCTION[1]

The value of vegetative propagation in forestry is well-understood, well-accepted, and well-documented (Zobel, 1981). Vegetative propagation of Eucalyptus has been summarized by Durrand-Cresswell et al. (1982), who list 118 references for the genus.

The transfer of non-additive characteristics is difficult through seed regeneration but is routinely possible when using vegetative propagation. Therefore, vegetative propagation is particulary attractive for achieving gains with characteristics that have very low heritabilities, such as growth and cellulose yields (Bonga, 1982; Jett et al., 1977). Vegetative propagation is especially useful for hybrids, making mass production of difficult-to-produce hybrids possible and using crosses among hybrids which have variable offspring (Zobel and Talbert, 1984).

For these reasons, Aracruz Florestal began vegetative propagation on a mass operational scale approximately five years ago, and now essentially all of the 12,500,000 trees planted each year by this company are vegetative propagules. Aracruz is now one of the largest and most successful users of vegetative propagation in Eucalyptus (Campinhos and Ikemori, 1977, 1980 and 1983; Campinhos, 1982).

It is the objective of this paper to summarize some of the gains and techniques that have enabled Aracruz Florestal to effectively use vegetative propagation on such a large scale.

THE SITUATION AT ARACRUZ FLORESTAL

Aracruz concentrates on Eucalyptus in its forestry operation, primarily producing wood for pulp (Anon., 1979). The growth conditions on the company's lands along the coast in the state of Espirito Santo are good; the climate is mild, and the soils are suitable, although not ideal, for forest tree growth. The mean annual rainfall of 1364 mm is well-distributed throughout the year, enabling the company to produce rooted cuttings and to field-plant all year. The land has gentle topography which facilitates mechanization.

The company's major product is high-quality, bleached sulfate pulp, much of which is exported to the foreign market. Therefore, quality standards for both wood and pulp are high and must be maintained if the company is to remain competitive. As a result of the need for high quality, Aracruz has a special interest in wood properties as they affect both pulp yield and quality; it uses vegetative propagation to meet these needs.

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[1] The paper is the summary of a talk based on slides; the written text covers only summary points from the slide presentation

The original plantations of Aracruz were mainly from the "Brazil grandis" source, which is very nonuniform, consisting of many trees that are inbred and contain numerous interspecific hybrids, some of which show a classical "hybrid breakdown" (Brune and Zobel, 1982). However, occasional, outstanding individuals, some of which appear to be hybrids, are found in these marginal stands, and Aracruz is using cuttings from these excellent trees in its current operational planting. Normally, these trees would not be used in a regular seed production or seed orchard program because of their hybrid nature (Durrand-Cresswell et al., 1982). Thus, the conditions for the use of vegetative propagation were nearly ideal for the development of Aracruz Florestal's program.

THE GENETICS PROGRAM

A regeneration program using vegetative propagation without a supporting genetics program to produce more greatly-improved trees will reach a "deadend" (Zobel and Talbert, 1984). After the very best trees in the current plantations have been located, no further improvement will be possible until the breeder develops even better trees which, in turn, can be used in the vegetative propagation program. This holds true for both pure species and hybrids. It is a fact that, generally, good hybrids can only be produced from good parents. This requires an intensive breeding program within the parent species before improved hybrids are produced. This concept of improving both the parent species and the resultant hybrids forms the basis of Aracruz's vegetative propagation program (Campinhos, 1980).

Aracruz Florestal has a special interest in Eucalyptus grandis and the E. grandis x E. urophylla hybrid (Anon., 1979; Campinhos and Ikemori, 1977). Large collections of E. grandis have been made by mother tree from the Atherton area in Australia and northward. Approximately 300 individual tree collections were made and are now being grown at Aracruz in mother-tree tests. From these, the most outstanding individuals are selected from the best families and placed in clone banks. The selected trees are crossed and tested, and advanced generation selections are made from the best families. Also, a number of breeding groups, each containing twenty trees, are being established from each of the best trees from the best 50% of the families using a modified sublining pattern (McKeand and Beineke, 1980). A similar, but less intensive program, is being used for E. urophylla. In the fourth, and possibly the third, generation, hybrids will be produced by using the best trees of advanced generation E. grandis and E. urophylla as parents. This will create a "super-hybrid" from the best of the advanced generation trees. The rather ambitious program outlined is not as unwieldy as it seems. The eucalypts at Aracruz flower rather profusely within a year and, since rotation ages are so short, selection for advanced crossing can be made at two-and-one-half to three years.

An important aspect of the Aracruz cloning program is that there is an intensive and ongoing genetic development program to provide even better material for the applied vegetative propagation program.

On a more limited scale, other genetic improvement is underway, such as crossing among hybrids. These do not breed true and the progeny of the crosses are variable. However, when hybrids are crossed, occasional individuals, which result from a chance combination of genetic material,are produced that are outstanding. These superior trees cannot be used in a seed program but they can be the basis for a vegetative propagation program after they have been tested for their value as clonal series of rooted cuttings.

DEVELOPING CLONES FOR OPERATIONAL USE

Similar to most forest trees, Eucalyptus must be in a juvenile state of development to be successfully propagated by rooted cuttings (Bonga, 1982). Juvenility is easy to obtain in the eucalypts; when a mature tree is cut, the sprouts produced at the stump show juvenile characteristics. Juvenile sprouts of all trees do not root equally well, and all trees do not sprout equally well. Those which root and sprout poorly are quickly eliminated from the program. This loss can be considerable. Therefore, it is essential to have a large number of selections. For example, Aracruz initially located 4,232 outstanding trees in its plantations. Of these, 614 were found to have desired phenotypes combined with suitable wood qualities. After reduction of numbers because of poor sprouting, rooting and performance in clonal tests, only 40 clones remained in the operational planting program. Only those clones which are most suited for each of the six major soil types that occur in the forest area of Aracruz are being selected. Adaptability of the clone to a given site is of the highest priority.

After the best phenotypes in the plantations have been selected and graded, the trees are cut. Those that sprout reasonably well are then rooted. If the rooting percentage is satisfactory (Aracruz uses 70% rooting as an acceptable level), as many individuals as possible are rooted from the cut stump and planted in clonal tests to determine if they possess the desired superiority and adaptability. The most outstanding clones from the tests are then chosen for use in the operational program.

METHODOLOGY

A detailed description of the methods used by Aracruz for producing rooted cuttings is described by Ikemori (1975, 1976) and Campinhos and Ikemori (1980, 1983). The following summarizes the methodology used:

1. The selected tree is cut approximately 12 cm above ground level. Coppice shoots arising higher than that (from about the fifteenth bud upward) do not often root well.

2. Coppice shoots taken for rooting must be of the right degree of succulence; if they are too woody or too succulent, rooting is not satisfactory. Only tests can determine the proper stage of

development in any one given region and for any species. New studies indicate it may be possible to use much of the succulent material that was previously discarded.

3. The shoots are severed about 2 cm above the stump and immediately placed in a bucket of water, shaded, protected, and transported to the preparation area.

4. The shoots are then cut into pieces (cuttings) with shears, each piece containing one or two pairs of leaves. The leaf area is usually reduced by severing off a portion of each leaf.

5. The bases of the prepared cuttings are immediately placed in fungicide; Aracruz uses Benlate.

6. The same day that the shoots are harvested and divided into cuttings, they are planted in the rooting receptacle containing a well-prepared and moist substrate. Aracruz has found vermiculite to be the best but other media are satisfactory. It has been found that media with considerable organic content, such as manure, or media such as peat, cause rotting of the cuttings even when Benlate is used.

7. The cuttings are treated with IBA (indolebutyric acid) that has been diluted 6 gr to 1 kg with talcum powder. Approximately 2 cm at the base of the cutting is treated with IBA. The cuttings are immediately inserted into the container and subjected to intermittent mist spray to keep the leaves damp.

8. Rooting occurs in two phases. The first phase lasts five weeks. Cuttings are kept in the shadehouse during this phase; they develop a good root system and initiate growth buds. In the fourth week, the seedlings receive 3 kg of NPK (5-17-3), dissolved in 100 litres of water. This makes a sufficient amount of solution to fertilize 15,000 cuttings and is applied by hand irrigation.

   The second phase starts in the sixth week and lasts five to seven weeks. The cuttings are removed from the shadehouse and placed in an adjacent area in full sunlight. They then receive another treatment with the same fertilizer listed above.

   At the beginning of the ninth week of rooting, selection takes place and consists of (i) discarding dead cuttings; (ii) separating live cuttings into large and small sizes; (iii) selecting one shoot from each and removing the remaining ones so there is a single leader; (iv) eliminating the original leaves after the cutting has produced a shoot; (v) removing any dead leaves remaining on the cuttings; and (vi) severing roots that may have grown through the base of the container.

   At the end of the tenth week, the large cuttings can be planted in the field. During the eleventh week, the smaller cuttings

are fertilized and during the twelfth week, they can usually be planted in the field. Overall losses due to death or deformity average approximately 15% of the original cuttings that were stuck.

9. The misting schedule is designed to continually keep the leaves moist until roots are formed at the base of the cuttings. There are a number of methods of misting with automatic or manual controls. The preferable method is to use intermittent mist propagation controlled by automatic equipment.

10. Any number of different types of containers can be employed. Aracruz uses a plastic container in a styrofoam frame manufactured locally. The container must foster root growth with good formation and assure contact between the rooting medium and roots of the cutting. Aracruz removes the rooted cuttings from the container, places them in boxes, protects them from the sun, and transports them to the field for immediate planting.

11. Preventative treatments with insecticides to control caterpillars may be necessary.

12. Two harvests of shoots are made in a season from each tree in the clonal multiplication area. The tree is cut at about 1.5 to 2.0 years of age. After the sprouts have grown to the proper size, all but two are harvested for use as cuttings. Two sprouts are left to grow to nourish the roots and subsequently cut to furnish additional cuttings. The ratio of clonal multiplication area to area for planting is 1:100. Therefore, to plant 100 hectares, it is necessary to have one hectare of clonal multiplication area from which cuttings are obtained.

RESULTS

Improvement has been obtained in growth rate, tree form, disease resistance and wood properties with clones using rooted cuttings (Campinhos, 1980). The gains achieved for wood improvement in Eucalyptus by Aracruz have been spectacular (Zobel et al., 1983). Growth superiority will continue to increase as clones are selected which are adapted to specific sites.

At Aracruz, growth rate in the original "Brazil source" plantings was about 36 m^3 ha^{-1} a^{-1}. With the first vegetatively propagated plantings, growth currently averages 64 m^3 ha^{-1} a^{-1}. Individual clones are under tests which produce over 100 m^3 ha^{-1} a^{-1} after five years of growth in the field. These clones will be used in future plantations. Future gains will not be quite as dramatic as the initial ones from the original "Brazil source" plantings. However, we are confident that some additional improvements, a portion of which will occur from a better matching of clones to sites, will take place. The unknown questions, for which there are currently no answers, are when will a limit

to the productive capacity of the site be reached, and how will the law of diminishing returns caused by limitations in moisture, nutrients, sunlight, or other, mask an expression of greater genetic improvement. Aracruz is aware of the limitations of productive capacity of any site and has employed the very best forest management practices of good site preparation, cultivation, and fertilization, which will enable the expression of the genetic potential. It is well-documented that maximum genetic potential can never be realized without also using the best management practices (Zobel and Talbert, 1984).

Clonal uniformity from rooted cuttings is remarkably good, with trees being straight and well-pruned. All trees within one clone at a given age and on the same site are approximately of the same diameter and height. The Eucalyptus canker disease (Cryphonectria cubensis[2]) is totally absent from vegetatively propagated clonal plantations although its infection varies from slight to moderately heavy in plantations established from seedlings. Aracruz has specialized in wood quality improvement. Wood is almost identical from tree to tree within a clone. The company has been able to increase wood specific gravity and cellulose yield per unit weight of dry wood by a significant amount while still maintaining the excellent form and growth rate of the trees (Zobel et al., 1983).

The genetically improved clonal stock has created some forest management problems. Rotation age had previously been six to eight years, but discussions as to whether this can be reduced to five years are presently taking place. If this is not done, the spacing in plantations has to be increased from the current 3.0 x 3.0 m to approximately 3.0 x 4.0 m. The fast-growing clones close their canopies rapidly and severe root competition sets in early. Growth rate is rapid; some clones average over 20 m tall at three years of age and 32 m tall at five years of age.

The age-old and controversial problems of numbers of clones to use and how the clones should be deployed had to be faced early by Aracruz Florestal. Although the methods used are still being discussed and some alterations may take place following further testing and assessment, the general pattern followed by Aracruz is to use about fifteen different clones on any given site (different sites will have differing clones) and to plant them in monoclonal blocks of ten to twenty hectares. Although the suitability of this approach can and will be debated for many years, Aracruz made the above decisions, based upon the best biological and operational information available. Changes will be made if and when data indicates the needs and benefits. From both a biological and operational point of view, the only logical deployment appears to be with monoclonal blocks. Experience has shown that intermixed clonal planting is neither biologically successful nor operationally feasible.

The above problems are of no serious concern. They have occurred as a result of the ability to economically produce clonal stock

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[2] Recently renamed from Diaporthe cubensis (Hodges, 1980)

rooted cuttings and on an operational scale with a large improvement in growth, form, wood qualities and site adaptability.

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CRYPTOMERIA IN JAPAN

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ABSTRACT

Sugi (Cryptomeria japonica D. Don) is one of the most important conifers in Japanese history. Artificial planting of sugi has a history of several hundred years. There are many seedling populations and cultivars which are propagated by cuttings, especially in southwestern Japan. The old cultivars originated from cuttings taken from natural layers or twigs, and are linked to shifting cultivation. These cultivars are still being used today and plus tree selection and other breeding activities are based on this material.

In the future, sugi cultivation will be promoted with adequate counter measures, such as genetic improvement, to combat the various destructive factors. A quality control system with epidemiological map information regarding these factors will hopefully be established.

RÉSUMÉ

Le cryptomeria (Cryptomeria japonica D. Don) est l'un des conifères les plus importants dans l'histoire du Japon. Sa plantation remonte à plusieurs centaines d'années. De nombreuses populations de plants et beaucoup de cultivars sont multipliés par boutures, surtout dans le sud-ouest du pays. Les vieux cultivars proviennent de boutures prises sur des marcottes naturelles ou sur des remeaux et sont liés au mode de culture itinérante. Ces cultivars sont encore employés aujourd'hui, notamment pour la sélection d'arbres plus et d'autres activités d'amélioration des arbres.

À l'avenir, la culture du cryptomeria sera favorisée grâce à des mesures appropriées, comme l'amélioration génétique, pour la protéger contre divers agents destructeurs. Un système de contrôle de la qualité utilisant des données cartographiques sur l'épidémiologie de ces agents sera établi, on l'espère.

INTRODUCTION

Since ancient times, Sugi (Cryptomeria japonica D. Don) has been one of the most important conifer species in Japan. It is believed that at one time, abundant sugi forests prevailed in the country. As sugi wood has a straight bole with soft wood which could be easily split even using the implements of the time, it was used for house construction, as well as many objects for daily use. Traditional Japanese houses were built using square pillars, ranging from 20.0 to 30.0 cm wide, and square beams and rafters to support the heavy roof. Sugi wood was suitable for the production of these square pillars because of its straight bole, fast growth, ease of processing, and nice colour and scent.

Today, the utilization of sugi wood is still as important. Moreover, sugi is easily regenerated by artificial planting of seedlings or rooted cuttings.

NATURAL DISTRIBUTION OF SUGI AND ITS GENETIC DIFFERENTIATION

The natural distribution of sugi is shown in Figure 1 (from Hayashi (1951) and Tsukada (1980)). By pollen analysis, Tsukada (1980) determined that during the last glaciation (about fifteen thousand years ago), the range of sugi decreased to refuge areas on Yaku Island, Wakasa Bay and Izu Penninsula, and possibly other areas as well. Yaku Island is famous for its huge sugi trees which are more than one thousand years old. Yaku sugi is believed to have isolated distribution even in this epoch. After the withdrawal of the glacier, sugi moved from the refuge areas to the north primarily, and now shows patch-like natural distribution (Figure 1).

Distributional genetic differentiation was clarified by Sakai et al., (1978) by analyses of needle morphology and peroxidase isoenzymes. They showed that one reproductive center of sugi is located in the area of Yaku Island, Yanase (Shikoku), and Owase (Kii Penn.), and an additional center is in snowy Tohoku (Akita). Ogiyama and Yasue (1981) analysed diterpenoids in the foliage of sugi, and classified natural sugi into the following four groups, namely, (1) Akita, (2) Hokuriku, (3) Shikoku and Kii Penn., and (4) Yaku Island. The results of Sakai et al. (1978) and Ogiyama and Yasue (1981) seem to be comparable.

A GENERAL VIEW OF SUGI CULTIVATION

It is believed that artifical planting of sugi began more than five hundred years ago in Kyoto district and other southwestern areas of Japan. Some feudalistic lords, especially in Kyushu, planted sugi to improve their finances. In Kyushu, sugi planting was closely linked to shifting cultivation. The lords owned the forest lands and the farmers were allowed to cultivate millets, buckwheat, taro, and other miscellaneous cereals for several years after cutting the forest and burning the vegetation. When they shifted to another site, they were

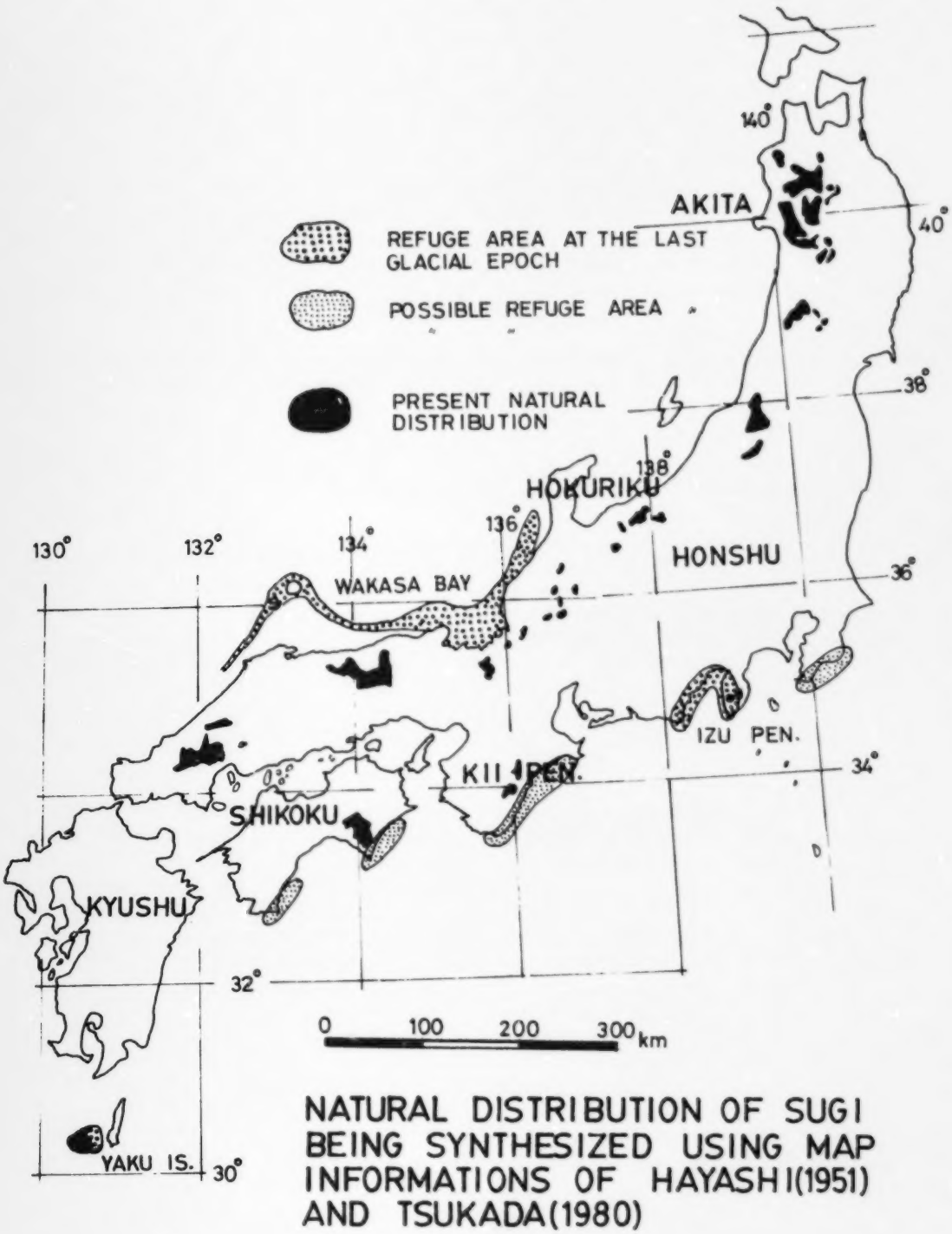


Figure 1. Natural distribution of sugi

(from: Hayashi, 1951 and Tsukada, 1980)

required to plant sugi in the old field. It is believed that these plantings were made mostly with direct cuttings from sugi twigs.

Miyajima (1983) produced a schematic distribution map of sugi showing both local populations which were propagated by seedlings, and local cultivars propagated by rooted cuttings (Figure 2). It should be noted that precise provenance testing of the seedling populations still has to be done and that the names given to the seedling populations in Figure 2 only represent their provincial or local names.

The general view of sugi cultivation in Japan in presented in several tables and figures. Table 1 presents the status of forest resources in Japan, while Table 2 illustrates the area of man-made sugi forests by age.

Sugi is the most widely cultivated species; 4.39 million ha (44.4%) of the total 9.895 million ha of man-made forest in Japan have been planted with sugi. (The second, most widely cultivated tree species is hinoki (Chemaecyparis obtusa Sieb. et Zucc., with 2.133 million ha (21.6%).) As illustrated in Table 2, most of the man-made sugi forests are still young - less than thirty-years-old. They are extensively planted and replace the forests of broadleaved tree species that were utilized for fuel and charcoal production after World War II.

Famous forest areas where sugi is cultivated are shown in Figure 2. The objectives of sugi production, length of rotation, planting density, and tending techniques are all different from each other. To better illustrate the concept of sugi forestry, an example of sugi cultivation is shown in Figure 3 (Funayama, 1983). In this case, the rotation period is fifty-five years, which is longer than the normal final age of thirty-five to fifty years. Sugi is planted with a density of 3,000 plants/ha, and is thinned several times to reduce the number to about 1,000 plants/ha at the final age. Mean tree height and mean diameters at breast height are 21.3 m and 28.0 cm, respectively. The bole volume is 580 m^3/ha, an additional 150 m^3/ha may be gained by thinning. Mean annual growth in volume is about 10 m^3/ha/a.

ESTABLISHMENT OF LOCAL CULTIVARS BY VEGETATIVE PROPAGATION

Toda (1974) described how the establishment of sugi cultivars was accomplished by taking cuttings from natural layers of indigenous sugi in Tizu, Sanin, and other places, and that clonal mixtures were used for regeneration. In Kyushu, in some cases, the scions of ancestral generations were taken from sugi groves located at religious places, such as shrines and temples. These groves may be the relics of natural sugi forests, or may have been introduced from other parts of Japan. The warm, humid climate, especially in the spring and summer, and the young age of the sugi plants from which the scions were collected, favoured the success in planting cuttings taken from sugi twigs. Shifting cultivation was also one of the forces assisting the creation of cultivars. The pressure placed on rooting was so strong that selection of ramets of single trees, or a group of trees with higher rooting ability, increased rapidly.

SEEDLING POPULATION

1. Ajigasawa sugi
2. Ooshuku sugi
3. Akita sugi
4. Todo sugi
5. Chokaimura sugi
6. Yamanouchi sugi
7. Makinosaki sugi
8. Honna sugi
9. Mura sugi
10. Kuma sugi
11. Tateyama sugi
12. Hakusan sugi
13. Hanbara sugi
14. Itoshiro sugi
15. Mumai sugi
16. Ashu sugi
17. Shiso sugi
18. Okinoyama sugi
19. Endo sugi
20. Hachiro sugi
21. Yoshino sugi
22. Yanase sugi
23. Yaku sugi

CULTIVAR PROPAGATED BY ROOTED CUTTINGS

(1) Sanbu sugi
(2) Clones bred by Mr. M. Fukuda
(3) Mura sugi
(4) Kuma sugi
(5) Boka sugi
(6) Ryowa sugi
(7) Betsusho sugi
(8) Masuyama sugi
(9) Kawaidani sugi
(10) Kanamidani sugi
(11) Tateishi sugi
(12) Mizumi sugi
(13) Hekosan sugi
(14) Hatsuta sugi
(15) Ichinami sugi
(16) Itoshiro sugi
(17) Nyukawa sugi
(18) Tane sugi
(19) Shiro sugi
(20) Shibahara
(21) Tomisu sugi
(22) Okinoyama sugi
(23) Endo sugi
(24) Tanami sugi
(25) Hachiro sugi
(26) Ichiki sugi
(27) Oki sugi
(28) Aya sugi
(29) Hon sugi
(30) Yabukuguri sugi
(31) Measa

Figure 2. **A schematic distribution map of sugi for both local populations being regenerated by seedlings and local cultivars propagated by rooted cuttings**

(from: Miyajima, 1983)

Table 1. Status of Forest Resources in Japan

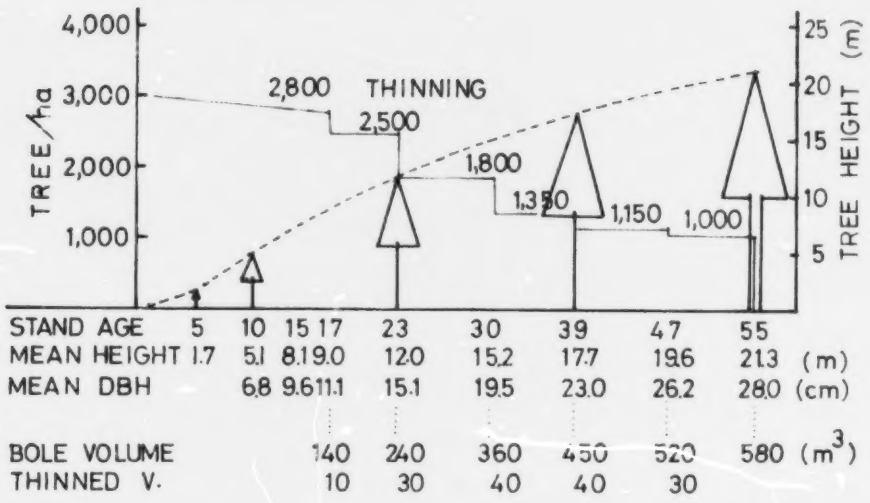
	Area (1000 ha)	Growing stock (Million m^3)		
		Conifer	Broadleaved	Total
Natural forest	13,994.0 (55.0%)	441.0	988.0	1,429.0 (58.0%)
Man-made forest	9,895.0 (39.0%)	1,041.0	13.0	1,054.0 (42.0%)
Other	1,390.0 (6.0%)	0.3	0.8	1.1 (0%)
Total	25,790.0	1,482.3	1,001.8	2,484.1

(from: Forestry Agency, 1982)

Table 2. Man-Made Sugi Forest by Age

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	Total
	(100 ha)													
National forest	758	1,092	1,551	1,229	1,057	765	222	134	210	250	175	134	291	7,868
Private forest	2,412	3,605	5,555	6,869	6,509	4,281	1,769	1,255	997	744	606	475	955	36,032
Total	3,170	4,697	7,106	8,098	7,566	5,046	1,991	1,389	1,207	994	781	609	1,246	43,900

(from: Forestry Agency, 1982)



AN EXAMPLE OF SUGI FOREST MANAGEMENT(FUNAYAMA, 1983)

Figure 3. An example of sugi forest management

(from: Funayama, 1983)

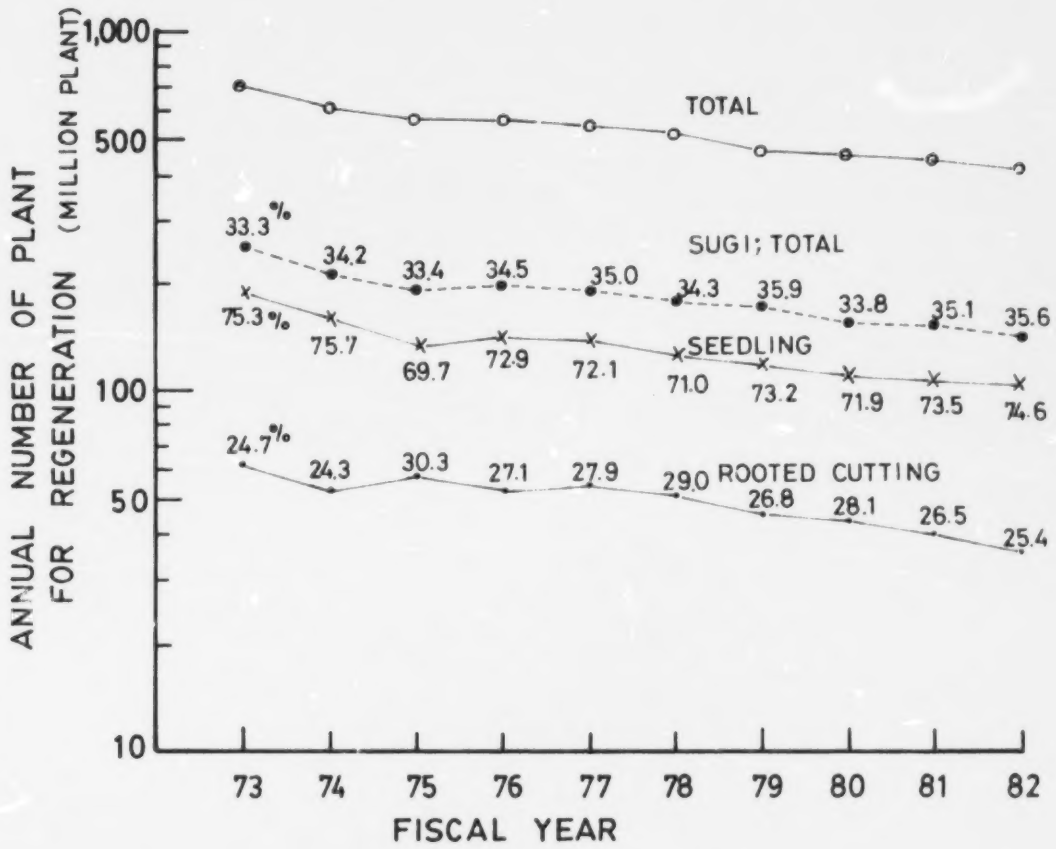
Phenotypic selection might have been made for fast growth, tree shape, bole straightness, and healthiness.

More than one hundred years ago, many local sugi cultivars were also bred by diligent foresters in several areas in Japan. At present, these cultivars are mostly regenerated by rooted cuttings.

COMMERCIAL PROPAGATION OF SUGI FOR ARTIFICIAL REGENERATION

The commercial production of sugi plants for artificial regeneration over the last ten years is presented in Figure 4. (The production in the National Forest is not included.) As a result of economic depression, regeneration areas decreased to about two hundred thousand hectares per year, on average and, therefore, a steady decrease in production has resulted. The ratio of sugi production to the total number of planting stock is about 35.0%. Rooted cuttings accounted for about 25.0% of the total sugi stock during this period. The mean production ratio of rooted cuttings of sugi for commercial use for each prefecture is presented in Figure 5. Production of rooted cuttings dominates in southwestern Japan, especially in Kyushu. This is comparable to the distribution of sugi cultivars shown in Figure 2.

Generally speaking, there are two modes for the production of rooted cuttings. In Kyushu, 30.0 to 40.0 cm long scions are rooted in open nurseries and after one year, the rooted cuttings are outplanted in



COMMERCIAL PRODUCTION OF SUGI PLANTS FOR ARTIFICIAL REGENERATION

Figure 4. Commercial production of sugi plants for artificial regeneration during the past ten years

(from: Forestry Agency, unpubl. data)

the forest. An outplanting mean of 70.0% or more is expected. In other areas, shorter scions (10.0 to 20.0 cm) are used and after two years, are outplanted. The difference in the mode of cuttings may be due to differences in climatic conditions between these areas; in Kyushu, it is warmer and, therefore, the growing season is longer. The scions were usually collected from so-called scion source forests - young forests which are regenerated by rooted cuttings of respective cultivars. However, in some cases, nursery stock was cultivated for scion collection.

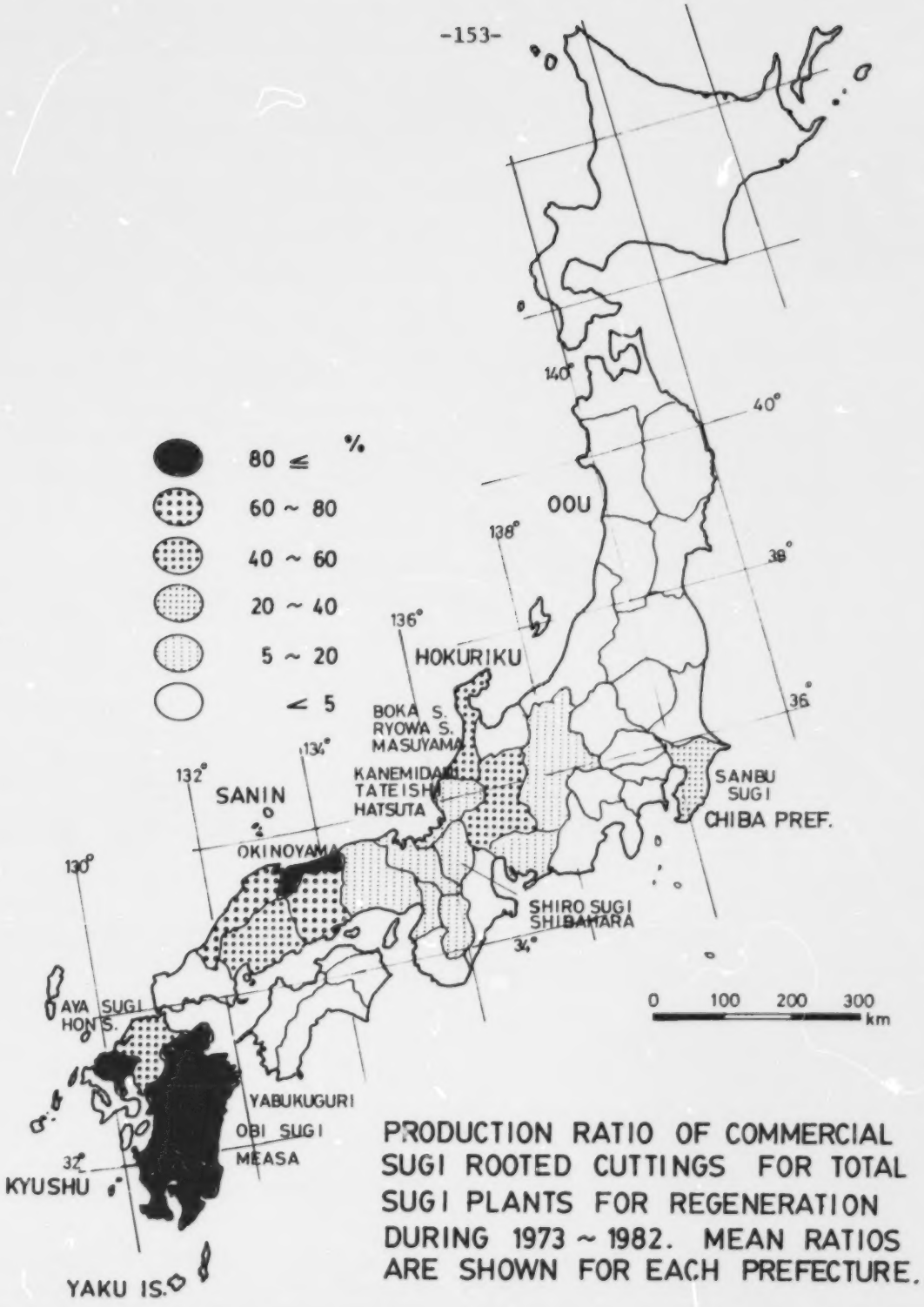


Figure 5. Production ratio of commercial sugi rooted cuttings to total sugi plants for regeneration during the past ten years. Mean ratio is for each prefecture.

(from: Forestry Agency, unpubl. data)

CHARACTERISTICS OF SUGI CULTIVARS

As shown in Figure 2, there are many local cultivars regenerated by rooted cuttings in practical forestry. Miyajima et al. (1979) described phylogenetic analysis of sugi cultivars in Kyushu. Measa, Hon sugi, Aya sugi and Yabukuguri have been proven to be old cultivars. Characteristics, such as growth habit (fast or slow; early or late maturation), straightness of bole, forking, heartwood colour (pink, red, black, intermediate), flowering habit (male, female and amount of flower or fruit, natural or artificial) have been closely investigated. In Figure 6, the height growth of four representative cultivars is shown. In Japan, site index is expressed by the mean tree height at forty years of age. In Table 3, Mashimo (1983) presents the quantification of growth (site index) of twelve cultivars for various environmental factors. Six characteristics, including cultivar, were classified into categories and using these, linear functions were established to estimate the site index. Soil type had the greatest influential effect on the site index, while cultivar was second.

The twelve cultivars were grouped as follows:

Growth	Cultivar
Very fast	Yabukuguri, Hon sugi, Urasebaru
Fast	Kuma sugi, Boka sugi
Intermediate	Ao sugi, Higo measa, Aya sugi, Obi sugi
Slow	Sanbu sugi, Okinoyama sugi
Very slow	Satsuma measa

PLUS TREE SELECTION OF SUGI AND OTHER BREEDING ACTIVITIES

In 1957, a nation-wide tree breeding program, by means of plus tree selection, was initiated in Japan. After ten years of selection, about nine thousand plus trees of different tree species have been registered. There are now about 3,600 sugi plus trees, 570.0 ha of seed orchard, and 522.0 ha of scion garden. For the selection of the sugi plus trees, it was decided that newly established cultivars (within the last fifty to one hundred years) could not be included as plus trees as these cultivars may resprout identical clones. However, many plus trees were selected from old cultivars with the aim of clonal separation.

Toda (1974) stated that at the onset of the work with sugi, it was planned to supply commercial forestry with rooted cuttings and a mixture of many clones. Vegetative propagation of sugi plus trees faced unexpected difficulty - the rooting of cuttings from the majority of selected plus trees was poor. Therefore, propagation of sugi was changed to the seed orchard concept, except in some districts such as Kyushu and Sanin where regeneration using rooted cuttings was common from old times. The most inexpensive method of producing rooted cuttings on a commercial basis is of prime importance to the growers. In some areas, a vinyl house and mist spray are used to produce higher rooting for the propagation of sugi plus tree clones. Plant growth hormones, such as beta-indole butyric

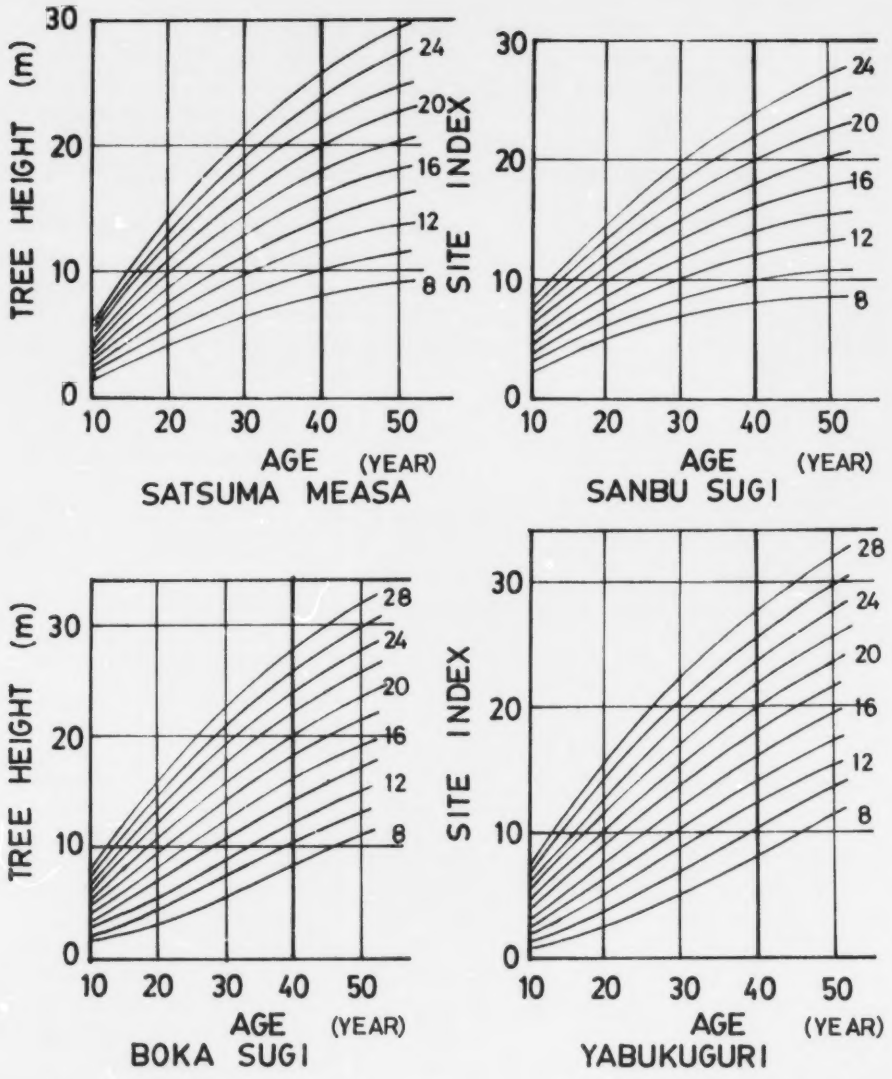


Figure 6. Height growth of four representative sugi cultivars

(from: Mashimo, 1983)

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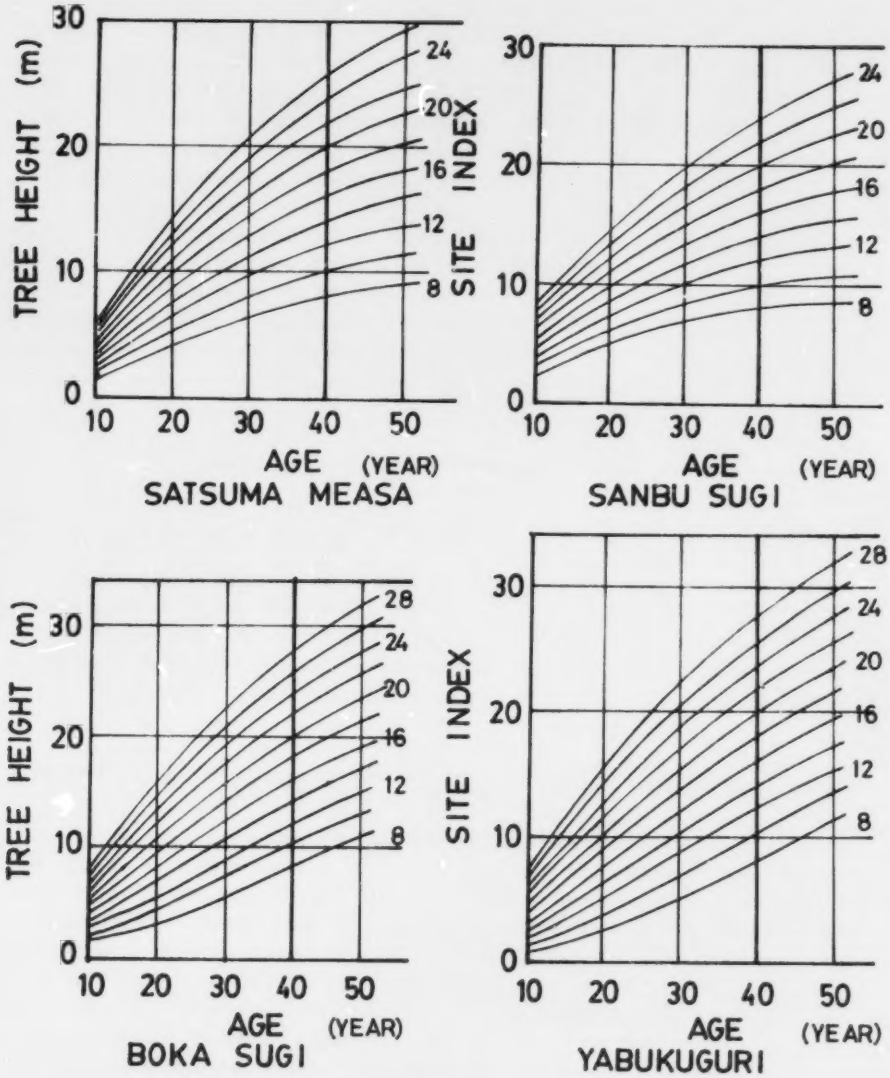

Figure 6. Height growth of four representative sugi cultivars

(from: Mashimo, 1983)

Table 3. Quantification of growth (site index) of sugi cultivars for environmental factors (from: Mashimo, 1983)

Factor / Item	Category	No. of stand surveyed	Mean site index	Score	Partial corr. coefficient and range
X_1, soil type	1. B_A, B_B, B_C	19	9.84	3.03	
	2. $B_{D(d)}$	43	14.26	7.567	
	3. B_D, deposit	21	17.19	9.451	
	4. B_D, creeping	68	20.15	11.893	0.885
	5. B_D, colluvial soil	37	21.95	14.172	11.997
	6. B_E	41	23.17	15.027	
	7. B1, plate	24	13.54	5.589	
	8. B1, creeping	18	17.94	9.822	
	9. B1, colluvial soil	13	21.00	12.338	
X_2, exchange Ca	1. 3 me/100 g, less	84	14.87	0.0	0.250
	2. 3-6 me/100 g	96	17.91	0.503	1.204
	3. 6 me/100 g, more	104	21.60	1.204	
X_3, geology	1. Volcanic ash	80	15.94	0.0	
	2. Andesite	51	21.00	0.398	
	3. Granite	41	16.78	0.016	0.340
	4. New sediment, rock	43	20.63	1.484	
	5. Old sediment, rock	57	18.75	0.949	2.284
	6. Serpentine rock, others	12	18.67	-0.800	
X_4, warmth index	1. 80 C, less	31	16.19	0.0	
	2. 80-95 C	82	18.54	0.771	0.259
	3. 95-110 C	122	18.93	0.618	
	4. 110 C, more	49	18.00	1.584	1.548
X_5, precipitation index	1. 140, less	27	16.26	0.0	
	2. 140-170	36	20.33	3.173	0.533
	3. 170-200	138	18.85	3.252	
	4. 200, more	83	17.37	3.502	3.502
X_6, cultivar	1. Satsuma measa	22	14.77	0.0	
	2. Obi sugi	37	18.05	2.392	
	3. Sanbu sugi	21	17.00	1.455	0.625
	4. Boka sugi	35	21.09	3.487	
	5. Kuma sugi	14	18.00	3.963	4.776
	6. Okinoyama sugi	17	15.71	1.364	
	7. Urasebaru	25	22.08	4.311	
	8. Aya sugi	38	17.26	3.234	
	9. Yabukuguri	25	19.46	4.776	
	10. Hon sugi	15	18.93	4.469	
	11. Higo measa	19	17.79	2.738	
	12. Ao sugi	16	18.13	3.227	

acid and its derivatives, are commonly used to improve rooting, especially for clones with intermediate rooting ability.

Another problem is a strong preference for homogenious phenotypes for planting material. Growers do not like to regenerate their forests with clonal mixtures which express different characteristics.

More than twenty years have already passed since the beginning of plus tree selection, and progenies of these plus trees are gradually being used for regeneration. During 1975-1979, early bred material of sugi was used for practical regeneration, such as seed collection on a scale of 12.0 to 45.0% and scion collection on a scale of 11.0 to 16.0%, compared to earlier seed or scion sources. Annual planting with this material was5.0 to 16.0% during this period.

We are now gathering the data from five- or ten-year-old progeny tests to determine the breeding effect of plus tree selection. As expected, a fairly good height increase is realized with these materials. Even for selection from cultivars, a gain was ascertained compared to local common cultivars. We are now developing an analytical technique on unbalanced data after grouping the testing plantations with varied clone or progeny composition. We are expecting to have the information on clone-site interaction to reconsider the breeding zone as well as the breeding value of each clone.

CLONAL ANALYSIS OF SUGI CULTIVARS BY ISOENZYME PATTERNS

Miyazaki and Sakai (1969) introduced the technique of isoenzyme analysis of sugi in Japan. Miyazaki and Miyajima (1981) made a thorough analysis of sugi cultivars in Kyushu on the basis of peroxidase isoenzyme patterns. They found that relatively few clones were mixed in each cultivar. They examined twenty-nine main native cultivars in Kyushu. Twenty-five of these showed distinct differences in the patterns, indicating genetic diversity. Further analysis within each cultivar was done, taking samples from large holy sugi trees growing around the shrines, temples, and other places scattered over Kyushu from which these cultivars seemed to have descended. The results were as follows: for Measa, eight different patterns in eight samples; Hon sugi - two different patterns in five samples; Aya sugi - three different patterns in five samples; and Yabukuguri - only one pattern in five samples. Judging from the peroxidase analysis, Yabukuguri, which has been widely used in Kyushu, is a single clone.

A similar type of analysis was made by Taira (1979) in Toyama district, and thirteen cultivars and one seedling population with a total of 2,736 plants were analysed on their peroxidase isoenzyme patterns. Variation of the patterns within each cultivar was two to less than ten. With these results, it may be concluded that some cultivars have less genetic diversity than expected.

OUTBREAK OF PESTS, DISEASES AND OTHER DAMAGE ON SUGI

In Figure 7, pests, diseases and other factors that could have destructive or fatal effects on sugi are schematically shown. According to climatic, ecological and other conditions, the destructiveness of each factor is variable. The necessity of counter measures will be determined by the degree of damage that is tolerable in each sugi forest management. As stated by Ohba (1979, 1983) and Ohba and Furukoshi (1981), in tree breeding, we are attempting to produce maps and tables showing isolines indicating the degree of damage resulting from the destructive factors. Although the information is essential for forestry management, the maps are not easily produced nor are they made with the same precision for each factor. Close cooperation with the scientists and technicians in various fields of forestry sciences and practices is needed in order to produce these maps.

Examples of fusiform rust outbreak in Pinus taeda and P. elliottii (Squillace and Wilhite, 1977) can be cited on maps of pests and diseases. Detailed survey maps on major forest insects and diseases are being published in a new format in Canada (Canadian Forestry Service, 1982).

Cowling et al. (1977) described a well-defined management strategy for southern pines to minimize the losses which are caused by fusiform rust thereby enabling decisions to be made for each step of pine forest management.

A simple plus tree selection program should be reconstructed if these fatal factors exist. With the information on a map, a decision can be made to (1) use the forest land for a purpose other than productive management, or (2) use the forest land for the purpose of forestry with the following counter measures applied alone or in combination: (a) silvicultural tending; (b) use of natural enemies; (c) use of chemical control; (d) genetic improvement; and (e) other (change of tree species, etc.).

The factors listed in Figure 7 were cited after a decision was made that the genetic improvement is of prime importance. At present, most of the sugi forests, except in higher elevations, northern locations, or in Kyushu, are potentially damaged by Semanotus japonicus Lacordaire (cryptomeria bark borer). Meanwhile, Resseliella odai Inouye (cryptomeria pitch midge) prevailed over all of Kyushu, originating from the Yaku Island during the last thirty years. Epinotia granitalis Butler (cryptomeria bark moth) is also expected to considerably damage sugi in Kyushu. These pests result in decay of sugi wood and will eventually kill the trees. Rooted cuttings are sufficiently resistant against needle blight (Cercospora sequoiae Ell. et Ev.). However, damage caused by other factors will not be greatly influenced with the mode of propagation, namely seedlings or rooted cuttings.

We are in the process of accumulating the information on varietal differences in resistance to several factors. For instance, Boka sugi and Yabukuguri show a high degree of resistance to Cryptomeria bark

Figure 7. A schematic presentation of pests, diseases and other factors that might have destructive or fatal effects on sugi in Japan

(from: Kanto Forest Tree Breeding Institute, Forestry Agency, April, 1981, unpubl. data. Rearranged by the author.)

borer. Yaichi and Obi aka seem to have a resistance to Cryptomeria pitch midge, while Yabukuguri is very sensitive to the insect.

The reasons for the recent occurrence of these damages are not clear. All of the factors are domestic, and slight damages have been noted for many years. As the damage in sugi forests has arisen from both seedlings and cuttings, it may be related to a wider cultivation of sugi - mono species cultivation.

A COMPREHENSIVE APPROACH FOR SUGI CULTIVATION IN THE FUTURE

As previously mentioned, sugi is very important in Japanese forestry. We have to deal with these fatal factors with foresight. As Libby (1982) reported, mixing of clones or seedling progenies may not be enough in some situations where the rate of resistant clones (or progenies) is low.

We are attempting to produce maps and tables indicating the limiting factors for forest management. A comprehensive approach for sugi cultivation is needed using map information, such as soil maps, climate maps, and vegetation maps. Epidemiological zonation of the destructive factors is planned, and the use of grouped plus tree clones with common superiority, or clones and cultivars which are resistant to certain factors should be the first step. The follow-up survey of plus tree clones (seedling progenies) is also very important. Forest damage may result from intricate interaction among many factors, such as the fatal factors, environmental condition, the genetic nature of the trees including aging effect, and silvicultural tending including planting density. Moreover, we need broader range of information of actual forests in "area" and not in "spot". It is hoped that a system of "quality control" similar to that which is common in industry, will be established. To make the system more effective, "a sensory system" (Ohba and Furukoshi, 1981) is proposed where small groups of test and control materials, with simple planting designs, are outplanted using the map information on regeneration sites with larger numbers. Follow-up surveys and calculation of the data will be made with the aid of a computer. This type of feed-back system is greatly needed in intensive forestry management.

With these ideas in mind, we are now conducting a pilot study on cross breeding of sugi in which combination and improvement of resistance against the fatal factors are expected.

Pest and disease conditions in forest tree species seem to be changing over a very long period of time (ten to fifty or more years). We need to be persistent in working for the control of these damages under a permissible level. The close cooperation among forest managers, research scientists and technologists in practical forestry is essential.

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THE EVOLUTION OF BLACK SPRUCE

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ABSTRACT

Morphological and genetic evidence indicates that black spruce is a relatively young species. It is thought to be of North American origin resulting from the hybridization of a proto-white spruce from eastern Asia migrating eastward, with a proto-red spruce of North American origin migrating westward. Differences in morphological characteristics between black spruce and red spruce, in the direction of white spruce, would indicate such hybrid origin. Today, it is difficult, but still possible, to effect a cross between a white spruce and a red spruce and produce hybrid seedlings which resemble black spruce.

Black spruce adapted to living in swamps and developed the ability to extract water from a soil solution of high osmotic pressure. It acquired resistance to forest fires and the capability to propagate vegetatively. Other features are the small cones, which are smaller than those of white and red spruce, and the dark purple colour of the immature cones.

RÉSUMÉ

D'après les données morphologiques et génétiques, l'épinette noire serait une espèce relativement jeune. Elle serait d'origine nord-américaine et proviendrait de l'hybridation d'une proto-épinette blanche de l'est de l'Asie migrant vers l'est avec une proto-épinette rouge d'origine nord-américaine migrant vers l'ouest. Des différences morphologiques entre l'épinette noire et l'épinette rouge, dans la direction de l'épinette blanche, indiqueraient une telle origine hybride. Aujourd'hui, il est difficile, mais encore possible, de realiser un croisement entre une épinette blanche et une épinette rouge et d'obtenir des plants hybrides qui ressemblent à l'épinette noire.

L'épinette noire s'est adaptée à un habitat de marais et a acquis l'aptitude d'absorber l'eau d'une solution de sol à forte tension osmotique. Elle a également acquis une résistance aux feux de forêt et la capacité de se multiplier par voie végétative. Elle a d'autres caractéristiques comme des cônes qui sont plus petits que ceux des épinettes blanche et rouge et qui lorsqu'ils sont immatures ont une couleur pourpre foncée.

Interest in planting black spruce on cutovers and burns, often on rich upland sites, has recently developed in Ontario. This interest is an outgrowth of earlier efforts in the Abitibi area.

The high wood quality of black spruce is well known. The densely-packed, long wood fibres are used as an admixture to improve other pulps for papermaking. In this respect, the black spruce pulpwood situation is similar to the Canadian hard spring wheat situation. It is easy to breed a spring wheat with more starch and less gluten and a greater yield as this requires less total solar radiation to produce a crop. However, the Canadian Wheat Board requires a certain minimal proportion of gluten in the grain before a lot is authorized for sale. Similarly, the selection of rapid-growing, so-called superior seedlings of black spruce could easily become counter-productive unless wood quality is considered.

Cloning of black spruce by mass vegetative propagation (Rauter, 1979), using very young seedlings as a source of stem cuttings, has shown promising results. Vegetative propagation of seedlings replaces the scarce seed supply of certain desirable provenances and increases nursery production. Other native spruce species, however, do not produce a sufficient number of new basal shoots which are suitable for cutting production after decapitation of the seedlings.

Morphological and genetic evidence indicates that black spruce is a relatively young species. There is no trace of black spruce in Asia. Swamps in eastern Siberia and Manchuria contain Larix dahurica Turcz. and related forms, but only dwarf Picea obovata Ledeb. and Picea ajanensis Fisch. (e.g. Ivashkevich, 1916; Kuzeneva, 1914). According to Linda B. Brubaker, the earliest fossil record of black spruce was found in the preglacial (Wisconsin) and in one of the interglacials (Brubaker, 1983 personal communication). Black spruce is thought to be of North American origin. It may have resulted from hybridization of a proto-white spruce with affinities to Engelmann spruce and Picea jezoensis Carr. from eastern Asia migrating eastward, with a proto-red spruce of eastern North American origin migrating westward, during a late pre-Wisconsin interglacial. At that time, there was abundantly available hybrid habitat and there must not have been any barriers to hybridization between these two species. Differences in morphological characteristics between black spruce and red spruce, in the direction of white spruce (Manley, 1971), would indicate such hybrid origin. Today, it is difficult, but still possible, to effect a cross between red spruce and white spruce (Gordon 1981) and produce hybrid seedlings which resemble black spruce (Gordon 1983 personal communication).

The Lakehead is a possible area of such past hybridization. Presently, black spruce contains morphological variation in both swamps and uplands in this area. The area is characterized as having had a continental climate, scattered flat muskegs, high latitude causing strong photoperiodic responses, and soil which was low in lime content (Laurentian Shield). These were all factors that may have favoured the initial establishment and evolution of black spruce. As a result, the putative hybrids may have evolved into the present-day black spruce. This

evolution may at first have been relatively very rapid following the pattern of punctuated equilibria, "A theory that postulates evolution as having occurred not as a series of continuous sequences of change but as a succession of rapid quantum changes interrupting the essentially static continuation of established species" (Stebbins 1982).

The new species adapted to living in swamps which previously represented unoccupied niches, as in eastern Siberia. It developed the ability to extract water from a soil solution of high osmotic pressure. It also acquired resistance to forest fires through a very peculiar female flower arrangement near the top of a tree with large clusters of old serotinous cones inside and green cones outside. During a fire, the green cones are sacrificed but the inner cones open and release seeds. The seeds can be several years old and requires only a moderate dormancy period to germinate. Often the individual tree is killed but its genes survive.

It also acquired the capacity to propagate vegetatively. A sphagnum bog is an alluvial habitat. The continuous growth of sphagnum moss chokes the lower part of the root system, and adventitious roots near the base of the stem and on buried lower branches are necessary for survival. All ofthese characters are not found to the same extent in the putative parent species and, therefore, must be the result of new gene combinations and mutations following interspecific hybridization. Other new features of black spruce are the small cones, a necessary adaption to fire, which are smaller than those of white and red spruce, and the dark purple colour of the immature cones which are darker than those of its putative parents.

In 1925, I was working for a pulp and paper company in Quebec at about 47°N latitude preparing a partially-burned forest area for salvage cutting during the following winter. In the unburned black spruce swamps, single, medium-large trees were marked and left as seed trees, and all others of merchantable size were harvested. The branches were lopped from the tops of the cut trees to favour rapid brush decomposition and thus reduce the hazard of future forest fires.

The area was revisited in the fall of 1928. It was found that nearly all the lopped branches were dark green in colour and lying in neat triangles on the ground with their tips turned upwards. Their basal parts were partially buried in live sphagnum moss as a result of past skidding operations, and had grown adventitious roots. They must, therefore, have been a type of non-juvenile rooted cuttings. The full use of these cuttings, for reforestation and breeding purposes, still has to be explored.

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REMARKS ON CLONAL FORESTRY RELATING TO THE QUESTION PERIOD WHICH FOLLOWED PRESENTATIONS AT THE CTIA CONFERENCE

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The following are some of the many questions surrounding clonal plantations:

1. Is there not a higher risk of disease and insect damage and poor ecological adaptation in clonal plantations?

2. Are there not more risks from all points of view in a large, genetically homogenous plantation (monoclonal or polyclonal)?

3. Should we continue to establish seed orchards and conduct traditional research for the genetic improvement of forest trees?

4. Despite the considerable benefits to be gained from the selection and reproduction of clones, will reforestation participants be willing to pay the additional costs involved in the production of trees derived from clones?

5. What silvicultural methods should be used in clonal plantations?

While some of the speakers at the conference gave examples of unsuccessful clonal plantations, others reported considerable success. What are we to conclude?

When we consider the failures attributed to clonal planting, we must distinguish between (1) those caused by an error in matching either clone to station, or even clone to environment, and (2) those caused by a high susceptibility to disease or insect damage of the clone or clones used in the plantations.

In the first case, the error is attributable to the silviculturist's poor choice or judgment and not to clonal planting, because the same error is made in the choice of species and sources in regular reforestation. The example cited by Dr. Libby of the I-214 poplar clone planted in Romania is representative of such an error. The I-214 was bred for ecological conditions in the Po valley in Italy, which has a sub-Mediterranean climate. This is probably very different from the climate in the region of the plantation described by Dr. Libby. The same errors are made when source trees are planted outside their hardiness zone.

In the second case, there is little justification for attributing the risk of disease or insect damage to clonal planting. Even in natural forests and regular plantations, considerable damage is caused by insects and disease. Some examples commonly found in eastern North America are the spruce budworm (Choristoneura fumiferana Clem.), which has destroyed 100 million m^3 of wood in Quebec alone; the Swaine jack pine sawfly (Neodiprion Swainei Midd.); the forest tent caterpillar (Malacosoma disstria Hbn.), which defoliates many deciduous species including trembling aspen and grey birch; the scleroderris canker (Gremmeniella abietina (Lagerb.) Morelet), which attacks red pine and jack pine plantations; white pine blister rust (Cronartium ribicola J C Fish), which is as common in plantations as it is in natural stands and on isolated trees; and Dutch elm disease (Ceratocystis ulmi (Buism.) C Moreau). Indeed, the presence of tree disease and harmful insects is very evident throughout the vast expanse of North American forests, which is one of the reasons why forest protection is usually a priority in the forestry policies of the countries concerned.

However, not all clonal forestry projects were considered failures. Two examples of success were Cryptomeria japonica, presented by Dr. Ohba, and the eucalyptus, presented by Dr. Zobel. Another success is the cultivation of poplars in Europe and the Middle East. Here, in spite of a limited number of clones, use of monoclonal planting, and the high vulnerability of certain clones to various insects and leaf and trunk diseases, the project has produced and is still producing large quantities of wood.

Therefore, while keeping in mind the limitations of clonal forestry, we must take all aspects into consideration and avoid unnecessary fears. We must recognize that insects and disease cause as much damage in natural forests as they do in regular and clonal plantations, especially when no attempt is made to select resistant or tolerant seedling stocks and when adequate silvicultural and protection methods are not applied.

Fears regarding the genetic homogeneity of clonal or polyclonal plantations are also unfounded, for although there may be genetic diversity in species and genotypes over the total area of a natural forest, within a given area there will be some degree of genetic homogeneity (through inbreeding of a given species). Furthermore, despite the diversity of species and genotypes, disease and insect damage can be quite severe in natural forests, as we mentioned earlier.

Through polyclonal planting, we will be able to group together very different genotypes of the same or different species whose silvicultural characteristics are similar (comparable growth rates) or complementary (tolerant and intolerant species).

The use of clones to establish plantations does not take away from traditional genetic improvement methods or seed orchards. For the time being, high-bred clonal stocks will be used only for intensely cultivated plantations so that their full production potential may be

realized. Furthermore, the genetic improvement of species is necessary in order to breed higher-performance clones of species or hybrids.

For the moment, problems with aging in clones of certain species (in particular, conifers) may be overcome, and production greatly increased, by taking micro-cuttings of seedlings from families selected for their high performance and raised in seed orchards. The production of polyclonal seedling stocks for reforestation ensures the continuation of seed orchards, because the production relies on the genetic advantages which the orchards can provide.

Production costs for clonal seedling stocks may be higher than for regular nursery stocks. However, the increased production which can be expected and the more intensive cultivation should compensate for the additional costs of clonal stocks.

There should be little difference between silvicultural methods used in clonal plantations and those used in regular reforestation. Experience with aspen, Cryptomeria japonica, spruce (in the Federal Republic of Germany), and various other species has shown that several silvicultural options are available. The uniform growth of clonal plantations would probably simplify thinning, as it could be done systematically.

In conclusion, clonal forestry will not replace traditional forestry; it is simply one more management option available to silviculturists for the production of wood. They must learn to use the option wisely in order to better meet human demand for wood fibre and products.

WORKSHOPS

1. PRODUCTION AND UTILIZATION OF GENETICALLY IMPROVED SEED

   – B.S.P. WANG AND D.A. SKEATES

2. ISOZYMES IN TREE IMPROVEMENT

   – W.M. CHELIAK

3. NORTH AMERICAN QUANTITATIVE FOREST GENETICS GROUP MEETING, 1983

   – S. FOSTER

1. PRODUCTION AND UTILIZATION OF GENETICALLY IMPROVED SEED

CHAIRMEN: B.S.P. WANG

D.A. SKEATES

PRODUCTION OF GENETICALLY IMPROVED JACK PINE SEED FOR PLANTING AND DIRECT SEEDING

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Keywords: Provenance, Gene pool conservation, squirrel predation

ABSTRACT

Bulk seed supply is no problem in jack pine, an important species in the boreal forests of Canada. Production of genetically improved seeds must be framed in the context of long-term management objectives and projected seed requirements for artificial regeneration of the species. Implementation of a seed production program requires consideration of local operational and biological opportunities and constraints, and the resource available over time. Each stage of development should produce the best seeds obtainable at that time, in the quantities needed and at an acceptable cost. A plan for seed production and progressive improvement of jack pine is schematically illustrated by steps from logging slash to seed orchard.

RÉSUMÉ

L'approvisionnement en vrac de semences n'est pas difficile dans le cas du pin gris, essence importante des forêts boréales du Canada. La production de semences génétiquement améliorées doit se circonscrire dans les limites des objectifs de l'aménagement à long terme et des besoins prévus en semences pour la régénération artificielle de l'essence. La mise sur pied d'un programme de production de semences exige qu'on tienne compte des occasions et des contraintes biologiques et opérationnelles locales et d'une projection réaliste des ressources qui seront exigées pour atteindre des buts précis. Chaque étape de l'exécution doit donner les meilleures semences possible, dans les quantités nécessaires et à un coût acceptable. Chaque étape du plan d'obtention des semences et d'amélioration progressive du pin gris est donnée, de la coupe des rémanents jusqu'au verger à graines.

INTRODUCTION

Jack pine (Pinus banksiana Lamb.) is a preferred species for reforestation by planting and direct seeding in the boreal forests of

Canada. In Ontario alone some 750 million seeds are required annually for planting (6%) and direct seeding (94%). Seed supply is generally not a constraint to artificial regeneration because of the abundant seed held in serotinous cones and ease of collection of cones from slash following normal harvest operations. As a consequence, foresters have several options available for immediate seed procurement and for planning future supply of jack pine seed. Alternatives range from buying cones with no control of origin to planning a continuing supply from designated stands, seed production areas or orchards. Their decisions will inevitably determine the genetic composition of new forests and, hence, the potential for their survival and growth as well as the genetic value of further generations that may be derived from them.

The purpose of this paper is to describe the range of levels of genetic improvement that may be applied to jack pine seed production and to indicate the resources required to achieve a targeted quantity of seed and genetic gain.

GENETIC IMPROVEMENT

Provenance

Provenance experiments have shown conclusively that survival and growth of planted jack pine is related to environment of seed origin. The evolutionary processes of natural selection, migration, mutation, and isolation have led to clear patterns of adaptive variation associated with latitude (photoperiod) and length and temperature of the growing season (Rudolph and Yeatman 1982).

Local seed collected from large, natural stands is the safest and best first choice in the absence of direct evidence to the contrary. For example, the local provenance did not differ significantly in height or volume from the first-ranked provenance at six out of seven test sites in Ontario (Table 1). The exception was at Caramat, north of Lake

TABLE 1 Ranks of mean-tree height and volume of local provenances growing in range-wide jack pine provenance tests planted at seven locations in Ontario

Test location	Site region	Ranks of local provenance	
		Height	Volume
Red Lake	4S	5	3
Thunder Bay	4W	5	2
Caramat	3W	20[1]	19
Fraserdale	3E	5	6
Swastika	3E	3	2
Twp. 114	4E	3	3
Petawawa	5E	6	5

[1] Statistically different at 5% level from provenance in first rank.

Superior, where at age 15 years western Quebec sources exceeded the local provenance in height by 9 percent and in volume by 36 percent. In identifying suitable local sources it is not sufficient that seed simply be from the same seed zone as the site at which they are to be planted. For example in site region 3E seed obtained from Fraserdale and Nellie Lake were planted near Swastika about 100 and 40 km to the south, respectively, but still in the same site region. Mean volume of the most northerly (Fraserdale) provenance was 18 percent less than the local (Nellie Lake) provenance.

Clearly, the first step in maintaining and improving the genetic quality of jack pine is to identify locally (within districts) large, well-stocked stands of good site quality representing a range of age classes. A diversity of such stands is needed for current seed collection and to implement a seed management program on the basis of forecasted requirements.

Selection

With control of provenance as a foundation, the next step in genetic management is improvement through selection and control of pedigree. Pedigree may be at the population (stand) level or it may refer to both stand and parent tree. The level of sophistication that may be applied, and hence the rate of genetic improvement and cost, ranges widely from simple designation of seed collection areas in advance of harvesting to development of complex improvement programs involving "plus tree" selection, seed orchards, progeny tests, breeding populations, and advanced generation breeding.

It should be emphasized that there is a substantial minimal cost for any tree seed. It is irresponsible management to accept the lowest tenders, to incur minimal collection costs or to offer marginal prices for cones or seed if the source of the cones/seed is unknown or if the source is known to be unsuitable. Cones/seed should only be accepted when location, number and type of stands and trees are specified and known within reasonable limits. Unacceptable collections include those from isolated trees, small isolated stands, poorly stocked or severely high-graded populations, unthrifty stands (low site quality) plantations of unknown origin, etc. Seed of low or unsuitable genetic potential will cost, in lost productivity, many times the savings that may accrue by emphasizing low cost of purchase or collection.

SEED PRODUCTION

Seed collection areas

Collection of jack pine seed from designated stands in advance of harvesting allows large collections to be labelled accurately with regard to origin. This label should remain with the seed and plants when returned to the forest. Subsequent performance can be monitored and knowledge of seed origin may be used to guide future seed collections from man-made forests. This strategy for seed production ensures that the

natural genetic base is maintained indefinitely at minimal cost. Substantial losses may be incurred in both the short- and long-term by using an improper seed origin or by moving seed beyond the limits of recognized ecological zones (Yeatman 1976).

Seed production areas

In areas (districts) where natural stands suitable for seed collection are severely limited, a continuing supply of seed can be assured by developing selected stands for seed production (Bitto 1977, Oldford et al. 1979). Such stands should be in the young to mid-rotation age classes. In this case, parcels of the designated stand are harvested only as required for seed production. The harvested area is regenerated with seed of the same origin to conserve the gene pool. Thinning of the stand several years before harvest will enhance flowering and increase the yield of cones per unit area. During thinning there is also an opportunity to upgrade the average quality of the trees left to bear seed by removing (roguing) all trees of poor form and low vigour. A further increase in seed production may be obtained by applying nitrogenous fertilizer to the thinned stand.

Seed production plantations

Plantations or regeneration from direct seeding of unknown or unsuitable origin should never be used for seed collection because of the risk of plantation failure or decreased growth caused by use of seed that are poorly adapted to the planting site or have an unknown, and possibly narrow, genetic base. Plantations that are known to be of local or preferred origin and to have been established from seedlings grown from bulked seed from a large number (e.g. > 200) of unrelated and non-inbred trees may be managed for seed production in the short term. In this context, part of an area successfully regenerated by direct seeding and/or by natural regeneration may also be a strong candidate for management for seed production. Thinning of the stand will enhance flowering and cone production. The even age and relatively uniform spacing in a plantation should reduce the amount of tree to tree variation in growth rate or form due to differences in the environment of individual trees. This increases the possibility that seed produced by the superior phenotypes left after thinning will give some genetic gain in traits of high heritability, such as branch angle and stem straightness.

A further level of genetic improvement may be obtained by establishing plantations from bulked seed of phenotypically selected trees (plus trees) and roguing within the plantations at a young age, say 8-10 years, after vigour and form have been expressed. In this way two levels of mass selection are applied to derive the production population.

Several such improved seed production plantations should be established within a given seed zone to ensure maintenance of a broad genetic base and reduce inbreeding over generations. A number of separate seed production stands are also required to provide insurance against disasters such as fire, tornadoes, insect epidemics, etc. At least 200 plus trees should form the genetic foundation of each population but preferably 500 to 1 000 seed parents should be represented. Each

population may be maintained for seed production and further improvement by repeated mass selection and re-establishment with bulked open-pollinated seed followed by roguing. This will provide seed with a satisfactory level of adaptation to the local environment and may give some improvement in the genetic quality of the seed at low cost, and with minimal dependence on specialist staff. Management for seed production does not preclude harvesting the wood.

Such populations could provide a sound foundation and interim backup for more sophisticated breeding programs involving seed orchards and progeny tests required to develop pedigreed breeding populations. The seed plantation by mass selection is the first generation of the type "B" orchard proposed by Yeatman (1979) for generating large quantities of progressively improved seed for direct seeding. This approach is also consistent with an interim, low cost scheme for genetic improvement recently suggested by Nienstaedt and Kang (1983).

Progeny tested seed orchards

Seed orchards are established for future production of genetically improved seed. Commonly, the seedling seed orchard approach has been advocated and applied to jack pine because of its early sexual maturity and natural fecundity (King 1973, Yeatman 1974, Klein 1974, Canavera 1975, Jeffers 1976, Kang 1980). A grafted orchard has been established in Saskatchewan (Orynik 1979, Roddy 1982). Factors of cost, graft incompatibility, slow initial development, absence of skilled grafters, and large targets for seed production have mitigated against grafted orchards for jack pine (Rudolph and Yeatman 1982). Seed orchards developed with genetically tested and selected cone bearing trees (seedlings or grafts) have been termed type "A" orchards by Yeatman (1979).

The design, development in time and space, and intensive management of such orchards and associated progeny/clonal tests is beyond the scope of discussion here. The relationship of type "A" orchards to type "B" orchards is illustrated in Figure 1.

Squirrels

Squirrels thrive in young stands of jack pine, commonly consuming virtually entire cone crops year after year. Thus, in spite of early sexual maturity, cone production in jack pine plantations may be disappointing if squirrel activity is not recognized and taken into account. The problem has not yet been faced in practical terms, but solutions will have to be found if potentials for seed yield are to be realized.

CONCLUSION

A program for seed production and genetic improvement must be set in the context of long-term management objectives and projected seed requirements for artificial regeneration of the species in question. Implementation needs to consider local operational and biological

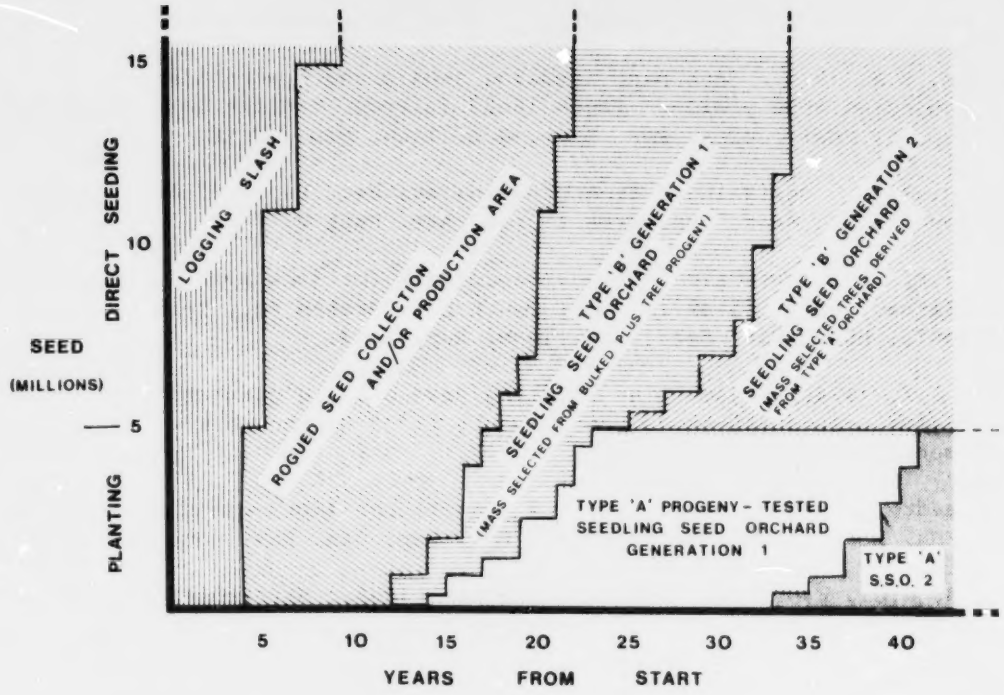

Figure 1. Projected supply and progressive genetic improvement of jack pine seed for a single component of a genetic management based on an annual requirement of 5 million seed for planting and in excess of 10 million seed for direct seeding.

opportunities/constraints and the resources likely to be available over time. Each stage of development should produce the best seed obtainable at that time, in the quantities required and at an acceptable cost.

A plan for seed production and progressive improvement of jack pine is schematically illustrated in Figure 1. Each step must also provide a sound genetic foundation for maintaining and improving the productivity of future crops. A well-planned program will ensure genetic and silvicultural stability and increasing economic value of the managed forest through successive generations.

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STRATEGIES FOR THE PRODUCTION OF IMPROVED SEEDS IN QUEBEC

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Keywords: Breeding, provenance, progeny

ABSTRACT

Quebec's annual target is 10 000 hl of cones to fulfill a regeneration programme of 100 million seedlings and 14 500 ha of direct seeding. Improved seeds are intended only for intensive forestry practices, i.e., plantations. Seed zones, based on forest regions, are defined within each administrative region and seed transfer is kept to a minimum, although superior provenances may be more widely dispersed when experimental results indicate this is advantageous.

Using numerous attributes, plus trees are selected in phenotypically superior stands from across the province; using grid lines no more than 1 tree per 0.5 hectare is chosen. A clonal seed orchard comprises 225 selections in contrast to 350 selections for seedling orchards (jack pine and black spruce). To date, 8 000 trees have been selected, 125 ha of seedling and 4.5 ha of clonal orchards have been established. All open-pollinated trees will be progeny tested. Provenance studies continue.

RÉSUMÉ

Afin de réaliser un programme de régénération de 14 500 ha au moyen de 100 millions de semis qui seront ensemencés directement, le Québec vise chaque année un objectif de 10 000 hl de cônes. Les semences améliorées ne sont destinées qu'à la foresterie intensive, c'est-à-dire, aux plantations. Dans chaque région administrative, on désigne des zones semencières, d'après les régions forestières, et les transferts de semences sont limités au minimum, même si les provenances supérieures peuvent être disséminées plus largement lorsque les résultats des expériences en montrent les avantages.

En se fondant sur de nombreux caractères, on sélectionne des arbres plus dans les peuplements de phénotype supérieur de toute la province; selon un quadrillage, on ne retient pas plus d'un arbre par demi-hectare. Le verger à graines clonal comprend 225 sélections,

comparativement à 350 sélections, pour le verger à graines de familles (pin gris et épinette noire). Jusqu'à ce jour, 8000 arbres ont été sélectionnés, et 125 ha de vergers à graines de familles et 4,5 ha de vergers clonaux ont été établis. Tous les arbres pollinisés librement seront soumis à des tests de descendance. Les études des provenances se poursuivent.

INTRODUCTION

The reforestation program for Quebec requires large quantities of seed. For the next few years, the regeneration objective is 100 million seedlings and 14,500 ha of direct seeding annually. This will require 10,000 hectolitres (Hl) of cones of various species each year.

Seed required for direct seeding, mainly jack pine (Pinus banksiana Lamb.) and black spruce (Pinus mariana [Mill] B.S.P.), are collected in seed collection or seed production areas. Improved seed from seed orchards will be used only for the production of either bareroot or container grown seedlings.

The strategies for the production of improved seed include: the use of seeds from already proven provenances and from seed production or collection areas, the selection of phenotypically superior trees, their reproduction in seed orchards and their evaluation in progeny and provenance tests for the generation of genetic information for the next breeding generation.

SEED AND BREEDING ZONES

Quebec is divided into 13 seed zones, established in the late 1960's. These are based on Rowe's (1972) forest regions and on climatic data. The resultant demarkation lines were adjusted to existing administrative boundaries such as administrative regions, townships or counties.

The breeding zones are identified by one or more adjacent seed zones within one administrative region. This corresponds to an area within which the seed can be moved freely and still remain adapted to the local soil and climate. The production of improved seed by various means, as defined later, takes place in each breeding zone.

SEED FROM TESTED PROVENANCES

In the province, the use of seed is guided by the general rule of thumb: local seed, i.e. seed from the same seed zone or part of a seed zone is safest unless other sources are proven better.

However, when results from provenance tests indicate superior growth characteristics, seed of some provenances or regions of provenances may be used outside their zone of origin. For example, white spruce

sources from the upper Ottawa valley and from the southern part of the province, south of Montreal, can be moved to other parts of the Great Lakes-St. Lawrence forest regions (Rowe 1972) which covers most of the St. Lawrence valley. The same holds true for eastern white pine (Pinus strobus L.) and possibly for other species. Tests have indicated that tamarack (Larix laricina [Du Roi] K. Koch) and black spruce seed can be moved to 1° north of their origin. Good quality Norway spruce (Picea abies [L.] Kast.) plantations have been identified in Quebec along with some excellent provenances from Europe and Russia*.

In some provenance tests up to 50% superiority in yield over the local sources has been demonstrated. Efforts are being made to collect seed from selected trees within these sources for immediate use in reforestation or for propagation in seed orchards. The best stands of such identified provenances are being managed as seed production areas.

SELECTION

For all species, phenotypic selection is conducted in each seed zone within each administrative region. Field staff are asked to locate and visit above average stands in their regions. Selected stands are mapped to ensure good distribution within given areas. Each stand selected is surveyed using inventory lines at 61 m intervals.

Plus trees are selected on the basis of such traits as height, diameter, taper, size, angle and number of branches, wood specific gravity, and resistance to frost and pests. Though procedures vary with species, the selection intensity in general is one tree per 0.5 ha of area. The minimum distance between two plus trees must be 61 m to avoid possible close parentage.

Each selected tree is identified by a number painted on its stem and its location in the stand is recorded by its distance and angle from a base line. The stand itself is identified on a map to enable further collection of material (seeds and/or scions). Dendrometric measurements are taken on the selected trees for further correlations with their progenies. Specific gravity will be determined on a wood sample obtained at breast height.

A minimum of 225 selected trees are required for the clonal orchards and at least 350 for seedling orchards (jack pine and black spruce).

To date, about 8,000 trees have been selected. Some of them are already represented in seed orchards and are being progeny tested. The species involved are mainly Norway, black and white spruce (Picea glauca [Moench] Voss), jack and white pine and tamarack.

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*Comite d'amelioration genetique des arbres forestiers au Quebec. 1983. Amelioration genetique des essences resineuses au Quebec: recherche et developpement. Manuscrit non publie.

SEED ORCHARD ESTABLISHMENT

Cones are collected from selected jack pine and black spruce trees at the time of selection or later, either by climbing or felling the trees. Seed is extracted in a newly established seed extraction plant in Duchesnay, near Quebec City. Basic information on number of cones and seeds per tree, weight of seeds per tree, number of seeds per cone, weight of 1,000 seeds, and the number of seeds per kg has been collected. The seeds are then stored in a cold room until needed for seed orchard establishment, progeny and provenance testing or other purposes.

Seedlings required for seed orchards and progeny tests are grown in Spencer-Lemaire containers. One hundred and twenty-five ha of seedling seed orchards have been planted according to established designs (Rauter 1978, Back 1983). Seedlings are planted in clusters of four, with six metres between clusters.

For clonal orchards, scions are collected from plus trees and grafted on rootstocks in greenhouses. The grafts remain in the nursery for two years before outplanting at 6 m spacing in the orchards. The first white spruce clonal seed orchard is scheduled to be established next spring. To date 4.5 ha of various species of larch seed orchards have been established. Before outplanting, two ramets from each clone are planted in a clone bank for production of additional scions, controlled crosses or other purposes.

The first generation seed orchards are established on a local basis on prepared sites. Site preparation for seedling seed orchards consists of clearing of slash by burning or crushing with a Marden and by cutting all stumps as close as possible to the ground. For clonal orchards, the site is cleared of all slash, stumps, rocks or other debris with a minimum soil disturbance. Site preparation takes place at least one year before planting.

Management of seed orchards includes control of competition and pests, fertilization and roguing.

PROGENY TESTING

All the open-pollinated trees represented in a seed orchard will be progeny tested. The information obtained will serve for roguing the orchards and the generation of genetic information for the next cycle of improvement. At present, one progeny test has been established with the same material used in the seed orchard and at the same time on a representative site for the species.

PROVENANCE TESTING

With the current program of systematic selection in the best stands, adequate quantities of seed from various sites and provenances are

becoming available for provenance tests. These tests are expected to provide valuable information on seed transfer. The identification of widely adapted provenances or regions of provenances is also expected from these tests.

SEED PRODUCTION AND COLLECTION AREAS

Some of the best young stands in many seed zones are managed as seed production areas. Young stands (natural or artificial) are preferred for their good response to treatments and for relative ease in cone collection. Management practices generally include: thinning, topping (jack pine and tamarack), and fertilization for increased cone production and pest control.

The best large natural stands in each seed zone (jack pine and black spruce mainly) are identified and reserved for cone collection from felled trees during good seed years.

Currently, above average stands are being reserved for seed collection. These will be cut to harvest seed when quantity and quality warrant. In the future, some stands will also be permanently reserved for seed production on the basis of their superior phenotypic quality as well as mean specific gravity of trees in the stand, determined from wood samples obtained from selected trees.

CONCLUSION

Much of the work described above is already well underway in Quebec and further steps will be taken in the coming years.

However, further development of tree improvement work will have to be undertaken to support or expand on what has already been done. Future needs include: improved grafting techniques, new or improved reproduction techniques for improved stock (cuttings, tissue culture, etc.), juvenile-mature correlations, means to accelerate seed production, means to control cone and seed pests in the seed orchards, and nutritional or cultural techniques to improve stock, etc.

A good start has been made in tree improvement activities in Quebec with the above strategies. These strategies may be adjusted as new information becomes available enabling us to increase yield and quality of seed in a shorter period of time.

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PROGRESS ON IMPROVED SEED PRODUCTION IN THE MARITIMES

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Keywords: Seed orchards, breeding strategies, Picea, Pinus, Larix

ABSTRACT

Tree Improvement programs are in place in all three Maritime Provinces. The cooperatives in New Brunswick and Nova Scotia have been effective in promoting tree improvement activities in each province. A total of 140.8 ha of seedling and clonal seed orchards has been established, comprised of black, Norway, red and white spruce, jack and white pine, and tamarack.

RÉSUMÉ

Dans les trois provinces des Maritimes, des programmes d'amélioration des arbres ont été mis sur pied. Les coopératives du Nouveau-Brunswick et de la Nouvelle-Écosse sont parvenues à promouvoir l'amélioration des arbres et, en tout, 140,8 ha de vergers à graines de familles et de vergers à graines clonaux ont été établis, constitués d'épinette noire, d'épinette rouge, d'épinette blanche et d'épinette de Norvège, de pine gris et de pin blanc ainsi que de mélèze laricin.

INTRODUCTION

Tree Improvement activities were first started in the Maritimes in the 1950's by the Canadian Forestry Service. Much of the early work consisted of establishing provenance trials of both native and exotic tree species, and studies of larch and spruce hybridization. It was not until the 1970's that applied tree improvement programs utilizing native species were considered, resulting from the expansion of planting programs and the expressed interest by provincial and industrial agencies.

By 1987, 350 million conifer seeds will be required annually to produce 90 million seedlings for the region's planting programs (Fowler 1979). The cost of producing genetically improved seed can be justified when reforestation programs are of this magnitude. To this end, two tree improvement cooperatives were formed in 1976 and 1977 respectively: the

New Brunswick Tree Improvement Council (NBTIC) and the Nova Scotia Tree Improvement Working Group (NSTIWG). The Prince Edward Island Forestry Service has set up a small program and relies on the Canadian Forestry Service for technical advice.

Membership in NBTIC consists of 10 industrial agencies, the provincial and federal governments and the University of New Brunswick. The Prince Edward Island Forestry Service is an associate member of NBTIC. The NSTIWG is composed of three companies plus the provincial and federal governments.

The purpose of this paper is to describe progress made in the Maritime Provinces in establishing seed orchards of various species for the production of genetically improved seed.

SPECIES PRIORITIES

The two species common to tree improvement programs in all three provinces are black spruce (Picea mariana [Mill.] B.S.P.) and white spruce (Picea glauca [Moench] Voss). Red spruce (Picea rubens Sarg.) is of particular importance in Nova Scotia and to a much lesser extent in Prince Edward Island, while tamarack (Larix laricina [Du Roi] K. Koch) is being dealt with by Prince Edward Island and New Brunswick. Norway spruce (Picea abies) [L.] Karst) and white pine (Pinus strobus L.) form a small part of the program in Nova Scotia. In New Brunswick, jack pine (Pinus banksiana Lamb.) is the fourth species receiving attention.

BREEDING STRATEGIES

The two basic breeding plans adopted are half-sib seedling seed orchards with accompanying family tests and clonal seed orchards with full-sib progeny tests. The plan chosen for a particular species depends on the genetic information available at the time. At present, the family tested seed orchard strategy is deemed most appropriate for black spruce and jack pine while the clonal seed orchard approach is considered to be best suited for white, red, and Norway spruce, white pine, and tamarack.

Long-range breeding strategies have been developed for black spruce (Coles 1981) and tamarack (Simpson 1983). These strategies may require modification from time to time as information from family and progeny tests and other studies becomes available.

SEED ORCHARD ESTABLISHMENT

The first seed orchards in the region were planted in the mid 1970's, but it was not until the two cooperatives were formed that establishment of orchards was greatly increased. There are 140.8 ha of seed orchards in the Maritimes (Table 1). The area established for each species reflects the particular breeding strategy chosen. There are some exceptions, however. The seedling seed orchards of white spruce in New

Table 1. Area of seed orchard by species established in each of the Maritime Provinces to the end of 1983

Province	Area (ha) of orchard by species										
	Black spruce		Red spruce		Norway spruce	White spruce		Jack pine		White pine	Tamarack
	SO	CO	SO	CO	CO	SO	CO	SO	CO	CO	CO
New Brunswick	62.0	0.5	-	-	-	8.1	0.7	37.0	0.4	-	1.5
Nova Scotia	6.0	-	-	2.9	1.1	3.6	2.2	-	-	1.3	-
Prince Edward Island	12.0	-	1.5	-	-	-	-	-	-	-	-

SO - Seedling seed orchard.
CO - Clonal seed orchard.

Brunswick and Nova Scotia were established using open-pollinated families originating from the Ottawa Valley. The purpose of this program is to develop good local sources and further improve "Ottawa Valley white spruce" for Maritime use. The red spruce orchards in Prince Edward Island were established from seed collected from selected trees. The clonal orchards of black spruce and jack pine in New Brunswick are part of one industrial agency's program and not that of NBTIC.

Most seedling seed orchards are planted on cut-over sites which are deemed conducive to seed production. Large sites are generally chosen to permit expansion over several years. Seedlings from the various families are randomly planted throughout the orchards. Clonal orchard sites have been selected on both forested and agricultural land. Since a more sophisticated lay-out is necessary for these orchards, a computer program developed by Bell and Fletcher (1978) is being used to determine ramet location.

THE FUTURE

Progress toward the production of genetically improved seed nas been made in the Maritimes Region, especially since the formation of the two cooperatives. In New Brunswick, seedling seed orchard establishment should be completed by 1987, but this is dependent on good cone crops, especially for black spruce. The planting of clonal orchards in Nova Scotia and New Brunswick will continue until the early 1990's. A clonal orchard of Norway spruce utilizing selections made from provenance trials may be planted in New Brunswick. Prince Edward Island will be planting a clonal orchard of tamarack and possibly one of white spruce.

All seed orchards in the region are presently too young to produce seed. Some trees in the small black spruce seedling seed orchard planted in 1978 in New Brunswick produced a few female flowers in 1982 and more in 1983. With efficient management practices and effective flower enhancement procedures, seed production should soon become a reality. By the year 2000, about 60% of the seedlings grown in the region should originate from genetically improved seed.

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OPERATIONAL COLLECTION OF SPRUCE SEED IN ONTARIO

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Keywords: Mechanical collection, cone harvester

ABSTRACT

Seed requirements for provincial silvicultural programs, and specifically for north-central and northeastern Ontario, are described. Operational collection techiques are compared. Although these related to general collections, the comparative cost data and evaluation of methodologies provide information relevant to collection of improved seeds.

RÉSUMÉ

Les besoins en semences pour les programmes sylvicoles provinciaux, plus précisément pour le Centre-Nord et le Nord-Est, sont décrits. Des techniques de récolte en grande sont comparées. Même si les données se rapportent à des récoltes générales, la comparaison des coûts et des méthodes valent pour la récolte des semences améliorées.

INTRODUCTION

The need for tree seed in Ontario is expanding to satisfy increased regeneration targets. A projected target for 1988 of 156 million trees for the planting program has been estabished. Of this 61% will be container stock.

As an example of this increased silvicultural activity, the Northern Region[1] of the Ontario Ministry of Natural Resources expects to plant 70 miilion trees in 1988. Of this, 9 million will be white spruce (Picea glauca [Moench] Voss) and 40 million black spruce (Picea mariana [Mill] B.S.P.), representing 70% of the region's target. Thirty million

[1] The Northern Region is comprised of the administrative districts of Chapleau, Cochrane, Gogama, Hearst, Kapuskasing, Kirkland Lake and Timmins.

of the black spruce will be container stock produced by the private sector. Most of the region's black spruce bare-root target will be accelerated transplants, started in the greenhouse and grown in government owned nurseries. Current plans are to produce white spruce trees as traditional bare-root stock.

Container stock and accelerated transplants require seed of high viability to assure efficient seed use. Despite this, spruce seed requirements are large. Over the next five years in the Northern Region, the spruce planting program requires 4,500 hectolitres of cones while direct seeding programs increase this by 2,500 hl to total 7,000 hl. The jack pine (Pinus banksiana Lamb.) program necessitates the collection of 25,000 hl of cones for a five year period. As in the rest of Ontario, cone collection in the Northern Region is a very active program.

The foregoing provides an example of the magnitude of the cone collection job which lies ahead. In organizing recent general collections of black and white spruce, several new methods were tried operationally in Northern Ontario. This experience is valuable and should be of assistance in future planning. This paper provides a review of these operational procedures.

OPERATIONAL PROCEDURES

Recent experience in the Thunder Bay area (1980) and in the Northern Region (1982) are the basis for this presentation. Cone crops in each case were good, and occurred at a time when there was insufficient seed in storage to meet nursery sowing targets. Because of this situation both cone collection programs were given very high priority. Various methods were introduced and operationally tested as methodologies for collecting both black and white spruce.

White Spruce

White spruce cones open soon after they reach full maturity. It is desirable to begin early to extend the collection period. Both collections were organized to begin as soon as the endosperm became firm, and the embryo was full, yellowish in colour and firm enough to be easily removed from a sectioned seed with the point of a knife. Cones, when laid on a desk opened within 24 hours instead of shrivelling. In Thunder Bay, this stage was reached and collections began on July 22, 1980. In the Northern Region cones were considered ready to collect by August 2, 1982. Cones were placed in cold storage within 48 hours of picking and artificially ripened by methods similar to those described by Winston and Haddon (1981). Both collections were essentially completed within two weeks, although cones on the trees remained closed for another month.

Seed from early collections had good germination when compared to the provincial average (Table 1). The best seed at Thunder Bay came from young trees free of cone insects, the poorest seed was obtained from mature trees infested with cone insects. Viable seed yield at Thunder Bay averaged 600,000 per hectolitre compared to 292,000 from the Northern Region. Spruce budworm was prevalent throughout the latter area and may have contributed to the reduced yields.

TABLE 1. Germination of white spruce seed from early collections in Northern Ontario

Location		Germination	
		Average (%)	Range (%)
Thunder Bay		82	70 - 94
Northern Region:	Chapleau	94	90 - 96
	Cochrane	93	85 - 98
	Gogama	75	---
	Hearst	87	77 - 94
	Kapuskasing	89	88 - 93
	Kirkland Lake	93	72 - 97
	Timmins	91	68 - 98
	Provincial Average	85%	

TABLE 2. 1982 Production rates and per unit costs of white spruce cones by method of collection

Method	hl/man/day	Costs/hl ($)	Remarks
Hydraulic boom lift	0.45	415	Hand picked cones
Hydraulic boom lift	0.83	375	Trees topped cone bearing slash machine processed in modified corn combine
Hand picked	0.80	150	Trees less than 20 ft. tall collected from step ladder on piecework basis
Fandrich Branch collector		271	Unsuitable for improved seed
Climbing tall trees to cut cone bearing slash	1.00	150	(a) Climber collected own cones on ground on piecework basis
	1.50	130-150 (estimated)	(b) Climber worked with ground crew to collect cones on piecework basis
TOTAL CONES COLLECTED:	Thunder Bay	519 hl.	
	Northern Region	5875 hl.	
YIELD PER TREE:	0.25 - 1.00 hl.		

Several methods were used to collect cones, resulting in different production rates (Table 2). The most successful was climbing and dropping cone bearing slash. The bulk of the cones were collected by this method, i.e. 375 hl at Thunder Bay and an estimated 5000 hl in the Northern Region. The climbing method was taught to Ministry staff during two-day courses held the week before operational climbing began. The technique used was free-climbing using safety belts with seat straps, and ropes as described by Yeatman and Nieman (1978). The training experience showed that there was no shortage of people willing to climb (It's fun!) even though about half were initially hesitant. The Northern Region collection, for the most part, was done by individual contractors on piecework. It took place during a period when most woods operations were shut down and unemployment was high. Reports from the districts indicated that almost all of these people climbed, without safety equipment, and topped trees to get cones.

Cone costs varied with method of collection. The least expensive cones (Table 2) were picked on a piecework basis by climbers dropping slash from tall trees or hand picking from 6 metre step ladders in plantations. These methods are efficient and favoured for operational collections. It is uncertain if people would hand pick cones in the crowns of mature trees for the same piecework rate because the time required to reach the top would lower production rates. The highest costs were incurred when equipment rental was added to the price of cones. Climbing to the cones is about as fast as using a hydraulic boom lift if

TABLE 3. 1982 Production rates and per unit costs of black spruce cones by method of collection

Method	hl/man/day	Costs/hl ($)	Remarks
Hydraulic boom lift	1.00	575	Tops collected, cones harvested in modified corn combine
Hydraulic boom lift		350	Hand picked $150 piecework, plus $200 machine rental
Top purchase ($0.75/top)		170	Cones harvested by machine
Hand picked cones from step ladders	0.14 0.6-0.8	630 150	MNR crew piecework
Hand picked cones from tops in recent logging slash		150	piecework
TOTAL CONE VOLUME COLLECTED:	Northern Region (in 1982)	5,460 hl.	
AVERAGE YIELD PER TREE:	1.1 l		

machine travel from tree to tree and set-up time are considered. Each piece of equipment supports only 1 or 2 pickers, so rental costs for large collections can be considerable.

Black Spruce

Black spruce cones do not open as readily, or as rapidly as white spruce and thus can be collected over a much longer time period. They are also difficult to remove from the branch. Collections in the Northern Region now take place from October to spring break in April or May. Unlike white spruce, black spruce are impractical to climb. Tall trees must be felled to gain access to the cones, or mechanical devices employed to elevate the cone picker to crown level.

Experience with different methods of picking black spruce cones is shown in Table 3. The available information suggests that hand picking at piecework rates is the cheapest way to procure cones. The involvement of mechanical equipment to elevate pickers, as with white spruce, significantly raises costs.

Relatively inexpensive cones can only be collected from trees 5 metres tall or from slash. The use of a modified corn combine to remove cones from slash is economical. However, the resulting seed is not suitable for use in producing container stock or accelerated transplants due to the high percentage of residual debris which cannot be removed using current seed cleaning methodologies in Ontario.

CONCLUSION

The collection of relatively large quantities of white and black spruce cones is required to meet regeneration targets in Ontario. Several collection techniques were successfully used and evaluated in two recent large scale operations. Early collection of white spruce cones was shown to have no deleterious effect on seed germination.

The experience confirmed that the use of machinery to assist in cone procurement resulted in higher costs than collection by hand. The application of a particular method in the future will depend on the local situation. Regardless of methodologies employed, given a good crop year, large collections are quite feasible, providing a high program priority and an appropriate level of planning, organization and training are included.

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OVERVIEW OF UTILIZATION OF IMPROVED SEED

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Keywords: Seed-quality, collection, handling, testing, storage, pre-treatment

ABSTRACT

A greater regeneration effort, which means many more seeds, will be needed if the forest resource is to be maintained. Improved seeds are expensive and must be used with care. Seed:seedling efficiencies vary from 18% to 45% so considerable improvement in nursery production can be obtained. Only 3% of all seeds used presently come from seed orchards, therefore seed quality must be maximized by stringent source selection, collections of mature seeds during good years only, careful processing and up-grading of seeds already in storage. Seed pretreatments to overcome dormancy and thereby stimulate the rate and uniformity of germination, the benefits of cold stratification, and the care of seeds prior to sowing are also important considerations.

RÉSUMÉ

Pour conserver les ressources forestières, il faudra consacrer un effort plus grand à la régénération, ce qui signifie davantage de semences. Les semences améliorées sont coûteuses et doivent être utilisées avec soin. Le rendement des semences en semis peut varier de 18 à 45%, ce qui laisse place à des améliorations considérables de la production en pépinière. Seulement 3% de toutes les semences utilisées actuellement proviennent de vergers à graines; la qualité des semences doit donc être maximisée grâce à la sélection rigoureuse des sources, à la récolte de semences mûres durant les bonnes années seulement, au conditionnement soigné et à l'enrichissement des semences déjà entreposées. Le prétraitement des semences pour surmonter l'obstacle de la dormance et par conséquent stimuler le taux et l'uniformité de la germination, les avantages de la stratification au froid et le soin des semences préalablement à l'ensemencement sont aussi des facteurs importants.

INTRODUCTION

Forest renewal is an important and urgent issue in Canada. If we do not increase our present regeneration effort, it will be difficult not only to support the existing forest industries but also to meet the nation's projected future needs.

Seed is the most important forest reproductive material for reforestation. The current regeneration program requires 4.1 billion viable seeds annually, and this will increase to 7.3 billion seeds by 1987 (Morgenstern 1979). The successful establishment of forests by planting and direct seeding is governed by three critical factors, quantity and genetic quality of seed, seedling production and establishment, and stand tending.

The seed we need for Canada's forest regeneration must be of high quality and in adequate quantities. At present and for some time in the future, the seed for our regeneration will come primarily from general collections identified by seed zones and from registered seed production areas. We have had seed orchard programs in a few regions of Canada for some years, but seed production is still very limited. It is expected that the estimated 7.3 billion viable seeds required to fill our regeneration goals in 1987 will come from seed production areas (55%), general collections (42%), and only 3% or 218 million seeds from seed orchards (Morgenstern 1979). Because of the scarcity of genetically improved seed, it is necessary for us to make the maximum use of the seeds available, that is to produce the greatest number of healthy shippable container seedlings or bareroot stock from a given number of sound seed. Until recently, seed efficiency, expressed as percentage of shippable bareroot nursery stock relative to viable seeds sown, has been very low. In Ontario seed efficiency varies from 18% to 45% depending upon age of stock and species among the 9 provincial nurseries (Skeates and Williamson 1979). Similar efficiencies in containerized seedling production result from sowing 2 to 5 seeds per cavity (Edwards and Huber 1982, Hallett 1982). As the cost of production of genetically improved seedlings is nearly as much as planting cost (Edwards 1981), it is good economics and sound forest management to improve our current practices not only in the collection, handling, processing and storing of seeds, but also in the efficient use of seeds.

This paper discusses some of the critical factors that affect the utilization of genetically improved as well as generally harvested seed for containerized seedling and bareroot stock production.

SEED QUALITY

Seed Source

One of the major requirements for reforestation of cut-over or burned forest areas is to regenerate them by seeding or planting with seeds or planting stock of proven adaptability to the sites. Although increased growth and yield can be achieved through the use of genetically

improved seeds, some of the greatest gains can often be realized by recognizing and practising the use of proper seed source (Nienstaedt and Snyder 1974). The danger of introducing inferior seed sources into new forests will not only affect the current generation but also subsequent ones (Yeatman and Morgenstern 1979). Local seed should be used unless adaptation of new seed sources is known, such as the superior growth of the Upper Ottawa Valley white spruce (Picea glauca [Moench] Voss) in northeastern United States and of the southern sources of eastern white pine (Pinus strobus L.) in the lower Michigan (Nienstaedt and Snyder 1974). When local seed is not available, alternate seed sources must be selected according to recommended rules for seed transfer or by seed zone designation (Morgenstern and Roche 1969).

Seed Collection, Handling, Processing and Storage

For most tree species, seed reaches its maximum quality at physiological maturity before harvesting. From that point on, maintaining that quality depends on the subsequent stages of seed handling, processing and storage. Any injury to seed from these stages of operation will influence their germinability, vigour as well as storability. The financial loss by mismanaging genetically improved seed will be many times higher than that of general collections.

In seed processing it is essential to develop and standardize operating kiln schedules and cleaning procedures for uniform quality seed production. One of the common problems in seed processing is the complete removal of empty seeds and resin. This factor is especially important for precision sowing and containerized seedling production. However, it should be realized that many sub-sized, under-developed or insect-damaged seeds can be removed with an air-screen cleaner or by a sizing screen; resin can be separated by flotation in water and empty seed can be removed by air aspirators, specific gravity tables or liquid flotation depending upon species. Most tree seed extraction plants now are equipped with gravity separators not only for up-grading coniferous seeds but also for southern hardwood seeds such as sycamore (Platanua occidentalis L.) and dewinged yellow poplar (Liriodendron tulipifera L.) (Bonner 1976).

The most interesting development in up-grading the physiological quality of tree seed is the Swedish technique known as IDS (Incubation-Drying-Separation) method which was developed for removing filled dead seeds from aged, stored seeds (Simak 1983). The IDS method is based on the principle that seeds, when imbibed with water and incubated at cool temperature for a period and then subsequently dried, will lose the moisture at different rates depending upon their viability. Apparently, the dead seeds have greater rates of losing moisture during drying and will float on the water surface (Simak 1983). This method, after refinement for various species, will be a valuable tool for up-grading aged, deteriorated seedlots.

Problems in seed storage usually involve high moisture content due to either improper conditioning during seed processing or improper storage conditions. Again, these problems can be easily overcome by developing and establishing standard operating procedures.

SEED TESTING

Testing of seed quality is an integral step of seed utilization. If seed quality cannot be assessed frequently, it should be tested at least on two critical occasions, i.e. immediately after processing and immediately before sowing (Bonner 1974).

Testing will evaluate the germination potential of the seedlots for calculating sowing rates and detect dormancy, fungus infection and other weaknesses of the seedlots. Although it is important to follow established national or international standards for testing seed quality such as the "Rules for testing seeds" of the Association of Official Seed Analysts (1981) or the "International rules for seed testing" of the International Seed Testing Association (1976), standard testing cannot be over-emphasized for practical operation especially for genetically improved seeds because of their value and scarcity. New and practical sampling size and testing procedures need to be developed for genetically improved seeds.

PRE-TREATMENTS OF SEED FOR SOWING

Treatment to Enhance Germination

As seedling development and growth is greatly affected by the speed or rate of germination, it is most important to recognize the type of dormancy and to prescribe an effective treatment for breaking it.

Among the common treatments, the most effective one to overcome physiological seed dormancy is moist, cold stratification or prechilling. There has been a misconception that moist, cold stratification could be detrimental to germination of non-dormant seeds. In our experience, we have never encountered such a situation, although we realize that some weak, deteriorated seeds in aged seedlots could be killed by cold stratification*.

Cold stratification will not only overcome seed dormancy, but also reduce the sensitivity of both the dormant and non-dormant seeds to their optimum requirements for light and temperature, resulting in increased rate and uniformity of germination (Figure 1). Cold stratification is apparently a standard seed treatment for white spruce, lodgepole pine (Pinus contorta Dougl.), eastern white pine and balsam fir (Abies balsamea [L.] Mill.) for containerized seedling production in B.C., Alberta, and the Maritimes (Hallett 1982, Mathews 1982). Cold stratification especially benefits spring sown seeds when the nursery soils are frequently too wet and too cold for untreated seed to germinate and develop properly (Lavender and Cleary 1974). For containerized seedling production, the period of stratification could be similar to the laboratory germination requirement, but it should be longer, perhaps twice as long as the laboratory germination requirements, for nursery sowing.

Dormancy of some species could also be broken effectively by application of growth regulators such as gibberellic acid (GA) and various

* C.E. Heit, personal communication, 1970.

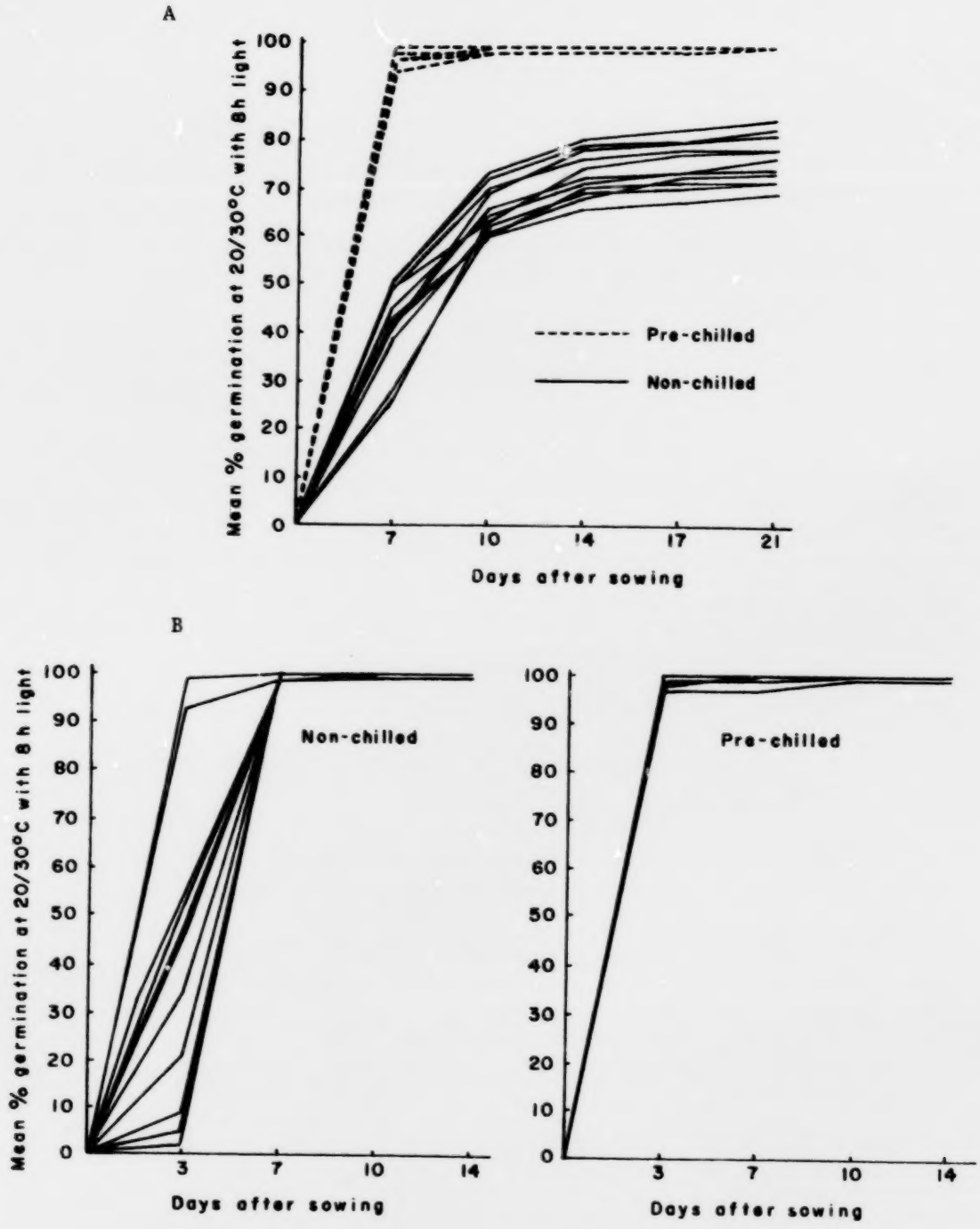

Figure 1. Effect of cold stratification on rate and total germination of monthly sown white spruce (A) and jack pine (B) seeds (Lines represent 12 monthly sowing times from April 1980 to March 1981).

cytokinins. A combination of GA (500 mg/l) and cold stratification can effectively break dormancy in basswood (Tilia americana L.) resulting in complete germination*.

Treatment of Seeds Against Fungal Pests and Small Mammals

This phase of treatment is usually part of the nursery operations and involves treating seeds with fungicides and rodent repellents at time of sowing. These treatments can be done by commercially available equipment in the forms of slurries, dusts or liquids as required (Bonner 1976). However, treatment with chemicals should be tested for any deleterions effects before mass application. For instance, the bird-rodent repellent, Arasan 42-S, which was found to have no ill effect on germination of yellow poplar seed (Belcher 1966), was detrimental to white spruce seed germination (Lamontagne and Wang 1975).

Pelleting of seeds has been a common practice with agricultural seeds for some time. It facilitates sowing operations of small-sized seed such as black spruce, as well as providing a means for applying desired chemicals or herbicides to germinating seeds. Little is known of the effects of pelleting on germination characteristics of seed under field conditions.

CARE OF SHIPPED SEEDS FOR SOWING

Seeds withdrawn from cold storage for sowing, especially those which are pre-treated, should be safely packaged in proper containers, clearly labelled, and shipped to the designated sowing sites by the quickest means possible. Upon receiving the seeds, field personnel should be instructed to handle such seeds with care by either sowing them as soon as possible, or storing them in a cool or refrigerated room until use.

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PROCESSING AND UTILIZATION OF SEED FOR PRODUCTION OF CONTAINER STOCK FOR REFORESTATION IN ALBERTA

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Keywords: Spencer Lemaire container

ABSTRACT

Seed operations in support of Alberta's Pine Ridge seedling production complex are described from seed extraction to seedling handling. Annual cone requirements are 7 000 hl of lodgepole pine and 3 000 hl of white spruce. The plant production target is 36 million bareroot and container seedlings.

RÉSUMÉ

Les étapes que subissent les semences destinées au complexe de production des semences de Pine Ridge (Alberta) sont décrites, de l'extraction des semences jusqu'à la manutention des semis. Chaque année, il faut 7000 hl de cônes de pin tordu et 3000 hl de cônes d'épinette blanche. On vise une production de 36 millions de semis à racines nues et de semis en récipients.

INTRODUCTION

In the past, forest nurseries in Alberta produced limited quantities of seed and planting stock for reforestation programs which relied heavily on mechanical site preparation and natural regeneration. Most of the forest tree seed and planting stock was produced over the past thirty years by the Department of Agriculture Provincial Tree Nursery located just outside Edmonton, Alberta. In 1976 the Alberta Forest Service began construction of the Pine Ridge Forest Nursery, which is located 140 kilometers northeast of Edmonton. Pine Ridge is Alberta's first forest nursery designed to produce economically, large quantities of high quality seed and planting stock for reforestation programs. The nursery currently processes and stores all seed for reforestation and produces 100 percent of all bare-root and about 80 percent of all container seedlings outplanted annually.

Unlike earlier systems, the seed processing and container seedling production at the Pine Ridge Forest Nursery are economically scaled and highly mechanized. They also incorporate recent scientific and technological advances. Both systems are specifically designed for white spruce (Picea glauca [Moench] Voss) and lodgepole pine (Pinus contorta Dougl.), Alberta's most important timber species. This paper presents an overview of each system's operations, technology and benefits.

SEED PROCESSING SYSTEM

Components of the seed processing system include: cone storage and drying sheds, extraction plant, cleaning plant, facility for cold storage of seed, and seed testing laboratory.

Upon arriving at the nursery, cones are unloaded into one of two steel-framed, screen-covered cone storage sheds. Each shed can hold 7,000 hectolitres of lodgepole pine or 3,000 hectolitres of white spruce cones. Lodgepole pine cones are stored in burlap sacks stacked on the floor of the sheds. White spruce cones are spread in stacking pallets to permit gradual curing and to prevent mold growth. For better control over the rate of cone drying and seed curing, white spruce cones are often spread on the paved floor of well-ventilated greenhouses where the temperature is held at 18°C.

The original design for the extraction plant was obtained from the Government of Saskatchewan, and modified for construction at Pine Ridge. Once operational, several additional modifications were made. The seed extraction plant runs 24 hours a day, five days a week, from November to February depending on the volume of cones to be processed. During this period it can process up to 18,000 hectolitres of cones. Continuous operation is energy efficient, maintains productivity and provides steady employment for local residents.

Lodgepole pine cones are forklifted from the storage shed to the extraction plant one day before extraction to warm them up to the base temperature of 16°C in preparation for scorching. The serotinous cones are funnelled through a pre-cleaning unit to remove flammable debris and soil particles before being moved by a shaker through the scorcher set at 210°C to 230°C for one minute. Scorcher temperatures are regulated for individual seedlots. It has been found that the degree of cone serotiny increases from north to south. As the cones drop out of the scorcher they are transported by a conveyor belt to holding bins where they remain up to one hour. Cones are then loaded into batch carts, moved over top of one of four individually controlled kiln rooms and fed by gravity into one of two tumblers. Lodgepole pine cones remain in the kilns for eight or more hours, depending on moisture content. The tumblers are controlled by intermittent timers set at varying intervals depending on species and moisture content of the cones. Intermittent tumbling reduces the amount of debris mixed in with the seed and improves the efficiency of the cleaning process. The extracted seed drops through the tumblers onto shakers which move the seed to collection bins located outside the kiln rooms. When the bins are full, the seed is bagged, labelled and moved to

the adjacent cleaning plant. By reversing the direction of rotation the empty cones drop down onto the shakers and are transported out of the kiln to a chute. From here they are moved by means of a vacuum system to a holding bin located outside the extraction plant for disposal.

White spruce seed extraction differs in several respects from that of lodgepole pine. The partially opened, relatively dry cones are collected in large wagons and moved to the extractor. They do not require scorching and are moved directly to the batch carts by an air-flow system. This ensures all seed that has fallen out of the partially opened cones is retrieved. Unlike the pine, the spruce cones require only three to four hours of tumbling at a kiln temperature of 40°C.

The seed cleaning system was developed by Hilleshög of Sweden and is the first of its kind in North America. In designing the system, Hilleshög recognized the economic impact of seed quality on seedling production, particularly on mechanized container seedling production. The objectives of the system are (1) to obtain seed of the highest possible purity and viability, (2) to reduce seed moisture content to a level conducive to long term storage and (3) to minimize mechanical and physiological damage during processing.

The seed cleaning process begins when the bagged seed is dumped into a hopper and conveyed up to the scalper. Here seed is first passed over an electronic vibrator to remove fine debris and cone scales. It is then passed over three screens of decreasing size to remove large debris and needles. The pre-cleaned seed is collected at the bottom of the scalper and manually lifted up to the wet dewinger. Wet dewinging minimizes the mechanical damage often experienced with dry dewinging. The wings are removed by spraying a small amount of water which breaks the bond between the wings and seed through differential expansion. A continuous stream of air blows the wings off the seed and out of the rotating drum. The dewinged seed is then passed through a screen to remove debris and fed by gravity into the liquid separator. Using water, the seed is injected 50 centimeters down into the liquid separator where damaged seed, resin and soil particles sink to the bottom and the remaining seed floats to the top.

An added feature of the liquid separator is that fungal spores and dust are washed off the seed coats and this has drastically reduced mold problems in seed storage and during germination. The seed which floats to the surface overflows into a pneumatic arm which distributes it over screen trays that pass through a positive control three stage dryer to lower its moisture content to the six to eight percent required for storage. Gradual lowering of seed moisture content eliminates seed coat case-hardening and physiological damage associated with rapid drying. On leaving the dryer, the seed is vacuumed up to a hopper which releases it at a constant rate over four screens of increasing size. This divides the seed into four fractions based on size, each dropped into the specific gravity separator where it is graded into three fractions of high qualiy seed based on weight and two fractions of empty and underdeveloped seed and debris. The three grades of high quality seed are sampled to determine purity, viability, moisture content and number of seeds per kilo-

gram. The seed is then heat-sealed in plastic bags and placed in waxed boxes for storage.

The cold room facility for seed storage is built into the side of a hill, 1.8 metres underground. The unit can hold up to 60,000 kilograms of seed in its four freezer compartments held at -18°C and 30 percent relative humidity. The underground design is energy efficient and provides protection against rapid loss of refrigeration and possible loss of seed due to fire.

CONTAINER SEEDLING PRODUCTION SYSTEM

Twenty greenhouses at the nursery can produce 20 million seedlings annually in two crops. The seedling production system consists of Spencer-Lemaire containers, a filling and seeding line, palletized crop movement, fiberglass covered greenhouses, and shade frames covered with removable Saran fabric.

The container production system was developed to use Spencer-Lemaire 40 cc Root-trainers. The book-like containers are made up of six cells per book and 17 books per tray for a total of 102 cavities. Both new and used containers, returned from the field, are used. The new books are assembled by local residents on a piece-work basis. The used containers are cleaned through a custom built washing machine and disinfected with dilute bleach.

The Alberta Forest Service designed, developed, and constructed a highly mechanized continuous flow filling and seeding line capable of filling and seeding 1.2 million cavities per day or 10 million seedlings in eight working days with 16 people. This reduces seeding time to a minimum and maximizes greenhouse time.

Container production begins at the dry mixer where the peat and vermiculite growing medium (2:1 by volume) is blended. The dry mix is then conveyed to the wet mixer where water and other additives such as wetting agents and pH adjustors are added. The moist mix is then moved by conveyor belt to two holding hoppers located over the start of two continuous-flow filling and seeding lines. The containers are placed on two-way conveyors that begin behind the holding hoppers. Vibrating agitators prevent the growing medium from clogging in the hoppers as the medium passes through an adjustable gate onto coarse sieves located at the bottom of the holding hoppers. The sieves remove debris and break up the soil mix as it drops into the trays moving over vibrating tables. The high frequency vibrating tables shake the medium into the cavities. At this point, a worker brushes off excess growing medium to ensure even filling of all cavities. As the trays leave the vibrating tables, rotating brushes sweep excess mix off the trays onto the lower conveyor belt which returns it to the holding hoppers. The trays then move under pneumatic packers which compress the growing medium down into the cavities and create seeding sites. As the trays move under the vacuum seeding heads, 2, 3, or 4 seeds are deposited in each cavity depending on the quality of seed being used. Container seedlings are usually grown from seedlots with 60 percent or better germination. However, in light of the high quality seed being produced by the seed program and increased production costs

associated with thinning and restocking empty cavities, the nursery prefers to use seedlots with 80 percent or greater germination. From the vacuum seeders, trays then move to the gritter where a thin layer of granite grit is deposited in each cavity to hold the seed down during germination.

Trays are then manually arranged with 16 trays to a pallet and forklifted or moved by wagon for a maximum of 500 metres to one of the greenhouses. The pallets are manually placed on moveable dollies. Palletization enables rapid tray placement and maximizes use of greenhouse space. The palletized crop remains in the greenhouses for 12 to 17 weeks. Growing parameters are set by a control panel located outside each greenhouse. Following the active growth period, the seedling crop is forklifted out the back doors of the greenhouses to the adjacent shadeframes. Here the crop is hardened-off and maintained until shipped to the field.

Seedling quality is matched to reforestation requirements by delaying shipping date until seedling specifications are met. The first crop is shipped in August of the current year or held over until May of the following year. The second crop is plantable by July of the following year, but usually shipped in August for fall planting.

With the desired technology and trained personnel in place, Pine Ridge Forest Nursery is concentrating on streamlining its operation to improve the quality of the seed and seedlings it produces, minimize seed wastage and reduce production costs.

PLANNED IMPROVEMENTS

Seed Collection and Processing

Pine Ridge is endeavoring to educate field foresters in the economic consequences of good and poor cone collections. A data base is being prepared to illustrate the effect of cone quality on processing and quality of seed. For example, in 1981, a poor white spruce seed year, processing costs were $63.11 per kilogram of cleaned seed with 68 percent mean germination. By comparison, in 1982, a moderately good seed year, costs to process fell to $30.67 per kilogram, in spite of 12 percent inflation. Mean germination of the 1982 seed rose to 81 percent.

Given the need to minimize seed wastage, Pine Ridge is studying ways to improve the economics of seed use especially for those seedlots having low germination and vigour. In light of the inability of the specific gravity separator to distinguish between dead and live seed of the same weight, Pine Ridge is investigating the application of I.D.S. floatation methods (Simak 1981) to remove the non-viable seed from a seed bulk prior to seeding. With upgrading seedlot quality, not only could poor quality lots be used in container production, but also the costs of thinning and restocking empty cavities, the greatest single production cost, could be decreased. Pine Ridge envisages use of upgraded seedlots with close to 100 percent viability for container production and is currently having a vacuum seeding head manufactured that will sow rows

with one seed per cavity alternating with rows of two seeds per cavity. The alternate rows will provide transplant stock if needed.

Seedling Production

Attempts to improve the size of the second crop by increasing the length of the greenhouse growing period are planned for 1984. The second crop will enter the greenhouses about two weeks earlier by double shifting the filling and seeding line. This will reduce the time taken to seed each crop from 10 days (including one non-productive weekend) to four continuous work days.

CONCLUSIONS

In conclusion, Pine Ridge Forest Nursery uses the best available technology for processing and utilizing seed in the large-scale production of container stock for reforestation in Alberta. This paper has presented a brief overview of the seed processing and container seedling production systems in place and of current efforts aimed at refinements which include:

1. Improvement of cone collections to decrease processing costs and improve biological seed quality.
2. Upgrading of low quality seedlots by removal of non-viable components.
3. Minimizing seed waste and reduction of production costs through single seed sowing.
4. Improvement of seedling size of second crop via increased growth resulting from reductions in time to seed a crop and longer active growing period in the greenhouses.

The systems in place and planned improvement should ensure that seed will be used most economically to satisfy Alberta's reforestation demands.

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IMPROVED SEED EFFICIENCY THROUGH HIGH DENSITY ACCELERATED PRODUCTION OF CONIFEROUS TRANSPLANTS

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Keywords: Pregermination, mini-containers, Picea mariana.

ABSTRACT

The essential goal is to reforest as many productive hectares as possible with the genetically improved seed available. Triple seeding of containers and the low seed efficiency of seedling production in open nursery beds will not achieve this goal.

Pregrowing or pregermination techniques for greenhouse production systems are discussed. Developments with the cigarette micro-container system at Maple are described with potential applications such as direct transfer to nursery beds as accelerated transplants, and for container production. Improved seed utilization is the principal advantage, but high density stocking in expensive greenhouse facilities, and the exploitation of natural conditions for large container production, resulting in more vigorous trees, are major considerations.

RÉSUMÉ

Le but essentiel est de régénérer, au moyen des semences génétiquement améliorées disponibles, les plus possible d'hectares productifs. L'ensemencement en triple dans des récipients et le faible rendement des semences en semis dans les planches de pépinières ne permettront pas d'atteindre ce but.

Les techniques de prégermination (ou de déclenchement prématuré de la croissance) destinées aux systèmes de production en serres font l'objet d'une discussion. Les progrès obtenus avec les micro-récipients à cigarettes, à Maple, sont décrits ainsi que leurs aplications possibles telles que le repiquage direct et anticipé dans les planches de pépinières et la production en récipients. L'utilisation des semences améliorées est le principal avantage de ces techniques, mais on retient surtout les possibilités de culture à haute densité dans les serres aux installations coûteuses et l'exploitation des conditions naturelles pour la production en gros récipients, qui donnent des arbres plus vigoureux.

INTRODUCTION

As we progress toward increased production of genetically improved seed, the essential goal will be to maximize the productive area restocked with fast-growing, high-quality trees. Seed in itself is of no value and its cost is relatively insignificant. The value of seed lies in its potential for production of forest products. That potential is determined the day the seed is collected, and is enhanced as we move toward registered seed of known origin, rogued seed production areas and seed orchards (Piesch and Stevenson 1976). Seed efficiencies of nursery systems of 10-40% in terms of shippable plants related to viable seeds sown, or greenhouse systems requiring triple seeding to ensure one seedling per container, will not be acceptable when genetically improved seed is used. Stock production systems with efficient use of seed will be essential.

The most efficient systems are those that provide optimum conditions at each stage of plant development. For example, the controlled environment germination cabinet is ideal for germination, ensuring a maximum yield of germinants. The greenhouse should be optimum for early plant growth, enabling the production of a maximum number of young seedlings.

The following is an outline of systems under consideration in Ontario using 'pregermination' techniques or 'pre-growing' systems (Skeates 1983). In a general sense, the intent is to use germinated seed, or plants in the early stages of growth instead of naked seed in production of coniferous planting stock.

PREGERMINATION OR PRE-GROWING SYSTEMS

Radicle Emergence Systems

Hagner (1981) developed a system in Sweden where seed germinates while floating on water supported by surface tension. As the radicle emerges the seed up-ends, breaking the tension and sinks to the bottom of the germinator where it is picked up by an underwater vacuum. Only germinated seed, with 1 to 2 mm of radicle are sown, either in inverted plastic cups established in the forest, or into stock production systems.

Fluid Drilling Inc. of Great Britain developed a system whereby seed is germinated in a temperature controlled, aerated water bath. Germinated seed is separated from non-germinated seed in a sugar solution. The efficiency of separation depends on specific gravity of the solution.

Sowing of germinants is accomplished in a ribbon of gel on seed beds in the nursery or in a water bath, distributed by vacuum, in containers. The Fluid Drilling equipment used in Ontario is designed to interface with a Dewa block machine which forms peat cubes.

Cotyledonous Plant System

At Maple, germination plugs were developed using a standard cigarette machine (Skeates and Williamson 1979a). A peat moss medium, the natural germination medium for black spruce (Picea mariana [Mill.] B.S.P.) in the forest, is used. An unwoven cellulose 'paper' similar to the mesh form of tea bag, encloses the cigarette which is cut to 1.5 cm lengths. A vacuum seeder distributes one seed to the top of each plug. Seed can then be incubated under optimum germination conditions. Successful cotyledonous seedlings are planted into peat cubes or other containers as soon as the seed coat is dropped and the radicle reaches the bottom of the plug. The Dewa block is seen as the most adaptable container for automation of planting germination plugs (Skeates and Williamson 1979b).

Mini and Micro Container Systems

A variety of small scale containers or pregrowing systems are on the market or under development. The purpose of each is to produce large numbers of plants at high density in high capital cost facilities such as greenhouses at a time of year when energy costs are also high. In each case the intent is to start the stock for use in subsequent systems. Two systems, the Blackmore 400 mini containers and the Blackmore waffles are felt to be more suitable for hardwood seedling production and therefore are not considered here.

Wiesinger's micro containers (Wiesinger 1982) are in a developmental stage, though they have been produced operationally, on a manual basis, for jack pine (Pinus banksiana Lamb.) containers used in Ontario's Ministry of Natural Resources Kenora District. Seed is sown in strips of corrugated plastic at 27,500 per square metre. Seedlings are removed and planted by hand though development of a mechanical transfer system is in the prototype stage. The very tiny cavity becomes a limiting factor in root development probably about the 6th or 7th week for black spruce.

Techniculture containers produced by Castle and Cook Inc. of California are soil medium plugs, similar to foam rubber in texture. Current products are available at 1400 and 3900 per square metre. The higher density is comparable to the Ontario tube. The system has been used very successfully in agricultural transplant systems in California and is currently being investigated for forestry use. The handling systems have been fully developed. The relatively low density of even 3900 per square metre results in high costs per plant. However the larger container size allows for a longer growing time in the greenhouse before it is necessary to move to a subsequent system for further plant development.

Cigarette micro-containers have been previously described in the cotyledonous plant system. The same cigarettes, produced in a continuous tube, are cut to 4.5 cm lengths to allow for growth beyond the cotyledonous stage to 10-week plants, at 8600 plants per square metre. After 10 weeks, roots appear to be restricted at this density.

DISCUSSION

Pregermination or pre-growing systems have their greatest potential in accelerated or greenhouse production. Economic studies* of Ontario forest tree production in greenhouses have indicated that plant density is the single most important cost factor in both capital investment and operating expenses. To make the best use of that investment, high density early production will be highly beneficial. The technique is considered for three stock systems.

Two Transplant System

To date most work in this field in Ontario has been with a two-transplant system using radicle-emergent seeds or cotyledonous germination plugs sown or planted in a container for subsequent transplanting into nursery beds. The first project, initiated in greenhouses at Maple, resulted in acceptable two-year transplants in Orono nursery when seed was germinated by the first of April (Skeates and Williamson 1979a). Early germination ensures that the optimum growing period in the year coincides with the normal stage of active plant growth.

Three pilot operations of 5,000 to 10,000 trees were conducted at Swastika nursery from 1977 to 1980 (Skeates et al. 1982). Pregermination was shown to be a significant factor in achieving conventional transplants, in a two-year system.

In 1980 a media/systems study was initiated at Swastika nursery to compare five soils or combinations of soils in three growing systems, bare-root, paper pots and Dewa blocks. The Fluid Drilling radicle emergent system was used. The use of cotyledonous plants in germination plugs is seen as providing greater benefits in terms of full stocking at a later stage of plant development, improved uniformity of plant and reduction in greenhouse time. All three systems proved capable of producing the standard of transplant required (Reese and Sadreika 1979).

Two alternatives are envisaged. The first is an early April sowing at high density in the greenhouse for planting in containers under shelters and subsequently summer transplanting. The stock is designated as 3G + 1-1/2, i.e. 3 months in the greenhouse plus 1-1/2 years in nursery transplant compartments. The second is a more promising summer sowing at high density to be overwintered, planted into a container and grown for a year and transplanted for one year in the nursery. This system, though requiring 2-1/2 years, should achieve a heavier grade bareroot transplant. The single year in nursery beds could double the capacity of spruce transplant production.

Single Transplant System

With the interest currently being shown in high density greenhouse systems for direct transplanting, i.e. a single transplant system,

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* Ernst and Whinney, 1982. Preliminary guidelines for the development of black spruce accelerated transplant systems. OMNR internal file report.

preliminary trials have been conducted at Maple. The first compared the Wiesinger containers with the 4.5 cm cigarettes for black spruce seedlings. At nine weeks the limited space in the corrugated plastic strips had restricted root development compared to the cigarettes. A second trial is currently under way in Longlac nursery of Kimberly-Clark with cigarettes and in Swastika nursery using Techniculture mini containers. As yet it remains to be demonstrated that the small container stock transplanted early in the season will achieve the necessary standard of plant for shipping in two years from the transplant beds.

If the single transplant system will achieve this goal, considerable economic benefit will accrue from the reduction in handling. It appears more likely that initiation of mini container stock the previous summer followed by dormant spring transplanting may result in achievement of desired standards.

Container Production

For container production the potential gains appear to be even greater. Plants produced at high density in the greenhouse can be transplanted into larger than normal containers outdoors where the cost of space is considerably less and where no heating energy is required. Present container systems represent a compromise between large units which allow optimum plant development but are costly in terms of greenhousing, and small units which limit plant development but are economically more feasible. Planting into containers outdoors in shelters early in June, to reduce the risk of late spring frosts, should result in large, healthy container stock for mid-summer planting.

In either the production of two-transplant bare-root accelerated transplants or of container stock, the peat cube would appear to be a promising system. The cube is formed without any cost of physical container. The mold can be designed to incorporate a dibble hole to accommodate the cigarette plug. The Dewa block system adapted at Maple from agricultural production has been modified for production of accelerated transplants in 2.5 cm cubes. Resources have been unavailable to mechanize transplanting systems, though concepts have been published (Skeates and Williamson 1979b). There is equipment on the market for production of large cubes on a production scale of over a million cubes per day in the Netherlands. The equipment should be adaptable for planting cigarette plugs instead of sowing seed.

CONCLUSION

Whatever systems evolve over the next few years, the economic advantages of time and space reductions in high density early plant development cannot be ignored. Though economic factors tend to weigh more heavily in the minds of North American foresters, by far the greater advantage will result from increased seed efficiency. The improved utilization of genetically improved seed will be a major tool in achieving our goal of rebuilding a future forest with vigorous, high quality trees.

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SEED EFFICIENCY IN HARDWOOD SEED PRODUCTION

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Keywords: Pregermination, accelerated transplants.

ABSTRACT

A target of 2.6 million trees, principally hard maple, soft maple, basswood, black locust and red oak, are produced by Ontario's four southern nurseries. An additional 400 000 hybrid poplar plants are raised from cuttings. Seeds are broadcast sown in greenhouse trays, and germinants are hand transplanted into Can. Am. containers. Savings in time and space have been considerable using this pregermination system. Germination under optimum conditions has resulted in higher seedling yields, very important when sowing with specialized seedlots and limited seed quantities.

RÉSUMÉ

Les 4 pépinières du sud de l'Ontario produisent, c'est leur objectif, 2,6 millions d'arbres, notamment de l'érable à sucre, de l'érable rouge et de l'érable argenté, du tilleul d'Amérique, du févier épineux et du chêne rouge. De plus, 400 000 peupliers hybrides sont obtenus à partir de boutures. L'ensemencement se fait à la volée, en bacs, dans des serres et les germinats sont repiqués à la main dans des récipients Can. Am. Grâce à ce système de prégermination, on économise considérablement de temps et d'espace. La germination dans les conditions optimales est d'un rendement en semis plus élève, ce qui est très important lorsqu'on ensemence des lots spéciaux et des quantités limitées.

INTRODUCTION

Difficulties have been encountered in reaching set targets of the traditional stock production types of hardwood seedlings in Ontario. Seed storage, stratification and germination are some of the factors contributing to these difficulties. Requirements for hardwood seedlings

Table 1 Sowing targets for principal hardwood species for 1984 in Ontario

Species	# of trees
Silver maple (Acer saccharinum L.)	600,000
White ash (Fraxinus americana L.)	550,000
Black walnut (Juglans nigra L.)	400,000
Red oak (Quercus rubra L.)	360,000
Black locust (Robinia pseudoacacia L.)	270,000
Sugar maple (Acer saccharum Marsh.)	100,000
Basswood (Tilia americana L.)	100,000

for the fast growing hardwood program in the Eastern Region have necessitated a more intensive cultural care to optimize the utilization of valuable seed.

PRODUCTION PERFORMANCE

The production of hardwood tree seedlings by the Ontario Ministry of Natural Resources has been confined mainly to the four southern provincial nurseries, namely Kemptville, Midhurst, Orono and St. Williams. Of the 3.6 million hardwood tree seedlings produced, about 2.6 million are standard regeneration species while the remainder are trees for wildlife habitat and for landscaping purposes. About 60 different species of hardwoods are grown. The targets for the major hardwood species in 1984 are listed in Table 1.

Besides the three main stock classifications from seed noted above, fast growing hardwoods for biomass production from cuttings represent a significant part of the Eastern Region program.

The achievement of production targets using hardwood seed has resulted in lower success rates than those experienced in coniferous stock production. Uncertainties in germinative capacity and germinative energy contribute to these results. Although it has been suggested that small seeded hardwood species can be stored for several years in the same way as most conifer seed, it has become apparent that seed viability declines rapidly (Aldous 1972, Schopmeyer 1974). Difficulties in breaking dormancy with many of the hardwood species result in germination occurring over a prolonged period of time, even up to three years. A tremendous variation in morphological characteristics of trees in the same age class results in high cull percentages at shipping time.

Besides production difficulties at the nursery, problems in plantation establishment contribute to restricted planting of these species. Hence the hardwood component of forest regeneration in southern Ontario is far below the levels desired by forest managers.

MODIFIED TECHNIQUES

To meet hardwood production targets it has been necessary to initiate growth in a greenhouse where greater environmental control can be achieved. During the last three years about 400 different seedlots have been grown at Kemptville to produce approximately 100,000 trees annually. In 1983, about 285 seedlots of Populus species, 57 seedlots of Alnus, 72 seedlots of Betula and 20 seedlots of other exotic fast-growing hardwood species were grown. Because many of these seedlots were very small and highly valued, quantities could not be sacrificed for testing purposes. To optimize the production from the limited quantities available, seed was carefully broadcast on trays of vermiculite and covered lightly with the same material. The seeded trays were then held at 22-24°C and misted frequently to promote even and complete germination. After germination was complete and primary leaf and lateral root formation was achieved, the seedlings were transplanted by hand into containers. Growth was maintained in the greenhouse until adequate sized plants were ready for transplanting into nursery beds when the weather was suitable. If transplanted in early June, trees of most of the fast growing hardwood species can be expected to attain a height of about 50 cm before growth stops in September. The trees are lifted in late fall and heeled in for improved access in early spring for shipment. Even with this intensive culture, and some culling at each transplanting operation as well as at shipping time, great variation in morphological characteristics can be observed at time of plantation establishment.

From modified treatments, derived from past experience and observation, it is apparent that great gains in production and outplanting success of hardwood stock can be realized. With genetic improvement in hardwoods, faster growth potential and better formed trees can be expected from future seed collections. Improvements in seed storage, stratification and germination techniques will help foresters to achieve that potential.

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2. ISOZYMES IN TREE IMPROVEMENT

WORKSHOP SUMMARY

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A half-day workshop on applications of isozyme technology in tree improvement and reforestation, organized by Dr. Bill Cheliak, was held on 22 August 1983. The program was organized around a lecture format and included both background information as well as applications.

The background information section included papers by Dr. Bruce Dancik (University of Alberta) and Dr. Peggy Knowles (Lakehead University). Dr. Dancik presented an overview of isozymes and introduced basic genetic concepts dealing with Mendelian genetics. Dr. Knowles elaborated on the technique of electrophoresis and discussed implications of the haploid/diploid nature of conifer seeds.

The application sessions were provided by Dr. Francis Yeh (British Columbia Ministry of Forests) and Dr. Bill Cheliak (Petawawa National Forestry Institute). Dr. Yeh's discussion centered on results and implications of studies of electrophoretic variation in natural populations. Dr. Cheliak discussed applications of isozyme technology for effective gene management of domesticated breeding and production populations.

Le 22 août 1983, un atelier d'une durée d'une demi-journée, organisé par Bill Cheliak, a porté sur les applications des isoenzymes à l'amélioration des arbres et à la création de forêts. Les travaux étaient de style conférence et comprenaient la communication de renseignements de fond ainsi que des discussions sur les applications.

Pour ce qui est des communications, M. Bruce Dancik (Université de l'Alberta) a donné un aperçu des isoenzymes et présenté les notions fondamentales de la génétique mendélienne, tandis que Mme Peggy Knowles (Université Lakehead) s'est étendue sur la technique de l'électrophorèse et a traité des conséquences de la nature haploïde ou diploïde des semences de conifères.

D'autre part, M. Francis Yeh (Ministère des Forêts de la Colombie-Britiannique) a discuté des résultats et des conséquences des études des variations électrophorétiques chez les populations naturelles, tandis que M. Bill Cheliak a traité des applications de la technique des isoenzymes à la gestion efficace des gènes de populations domestiques destinées à l'amélioration et à la production.

3. NORTH AMERICAN QUANTITATIVE FOREST GENETICS GROUP

ABSTRACTS

PROGENY TESTING EASTERN WHITE PINE: SECOND-FIELD-YEAR DATA

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ABSTRACT

Open-pollinated white pine seedlings from 60 different seed sources showed highly significant differnces in their second field season's growth at New Brunswick, N.J. Forty-nine of the sources were selected clones in seed orchards; within this group, mean growth of seedlings from the best clone, Maryland No. 8, was 46% greater than the plantation mean and 122% greater than mean growth of the poorest clone, Minnesota No. 27.

RÉSUMÉ

Des semis de pin blanc obtenus, par pollinisation libre, de 60 sources de semences ont fait preuve de différences très significatives au cours de leur deuxième année de croissance en plein champ à New Brunswick, au New Jersey. Quarante-neuf des sources étaient des clones choisis dans des vergers à graines; dans ce groupe, la croissance moyenne du meilleur clone, Maryland n°8, était supérieure de 46% à la moyenne de la plantation et supérieure de 122% à la moyenne du clone le moins performant, Minnesota n°27.

TO TRANSFORM OR NOT TO TRANSFORM

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ABSTRACT

Interpretation and prediction of tree improvement program results may be improved or hindered by the use of data transformation. In a white ash provenance test, logarithmic transformation of height growth reduced provenance by plantation interaction and stabilized error variance but gave poor prediction for the best seed source. Arcsine transformation or survival data yielded a poor fit to the response surface. In a black walnut twin study, environmental variance and correlation between ages can be better estimated with square root transformation. General methods of selecting a transformation are discussed.

RÉSUMÉ

La prédiction et l'interprétation des résultats des programmes d'amélioration des arbres peuvent être améliorées ou empêchées par la transformation des données. Dans un test de provenance sur le frêne blanc, la transformation logarithmique de la croissance en hauteur a réduit l'interaction de la provenance par plantation et stabilisé la variation de l'erreur, mais a fait en sorte que la prédiction de la meilleure source de semences a été mauvaise. La transformation en arc sinus des données sur la survie a donné un adjustement faible à la surface de la réponse. Dans une étude-soeur du noyer noir, la variance due au milieu et la corrélation entre les âges peuvent être mieux estimées par la racine carrée. Les méthodes générales de sélection des transformations font l'objet d'une discussion.

OPTIMUM ALLOCATION OF EFFORT WHEN INFORMATION IS AVAILABLE FROM FAMILIES, GENOTYPES, AND RAMETS

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ABSTRACT

Simple gain formulas for selection among and within full-sib families were modified to include the effect of clonal replication on forward selection for GCA and selection of a propagation population. Total gain, as the sum of gains from among and within family selection ($G_T = G_B + G_W$), was calculated under a variety of genetic conditions including mating designs and genotype/ramet allocation, but with constant population size. Optimum allocation was indicated where G_T was maximized.

The optimum allocation for a fixed population size depends on the proportion of families and numbers of individuals saved, on the heritability, and on the ratio of additive to dominance variance. There were different trends evident within the different mating designs. General results can be summarized as follows. For GCA forward selection, the range was one to six ramets, and the most frequent optimum, considering all sets of parameters, was two ramets. Within a given mating design, the optimum number was highest (six or four) with low family selection intensity and a high V_A to V_D ratio, and was lowest with high family selection intensity and low V_A to V_D ratio.

Interpretation of the results should be weighed against the limited set of population sizes and genetic parameters used. Further analysis of this question, which includes half-sib information in tandem or combined (index) selection, is called for.

RÉSUMÉ

On a modifié les formules simples du gain pour la sélection interfamiliale et intrafamiliale de pleins germains en y incluant l'effet du clonage sur la sélection améliorante pour l'aptitude générale à la combinaison (AGC) et la sélection d'une population destinée à la propagation. Le gain total, c'est-à-dire la somme des gains de sélection interfamiliale et intrafamiliale ($G_T = G_B + G_W$) a été calculé pour une variété de conditions génétiques y compris les schémas d'hybridation et le partage de l'effort entre les génotypes et les ramets, mais avec une population aux effectifs constants. Le partage de l'effort était optimal lorsque G_T était maximisé.

Le partage optimal pour un effectif constant dépend de la proportion de familles et du nombre de sujets retenus, de l'héritabilité et du rapport de la variance additive à la déviation de dominance. Selon les schémas d'hybridation, on a constaté des tendances divergentes. Les résultats généraux peuvent se résumer comme suit. Pour une sélection améliorante de l'AGC, la gamme était de un à six ramets, et l'optimum le plus fréquent, compte tenu de tous les ensembles de paramètres était de deux ramets. Pour un schéma donné d'hybridation, le nombre optimal était le plus grand (six ou quatre) avec une sélection familiale faible et un rapport V_A/V_D élevé et il était le plus petit avec une sélection familiale forte et un rapport V_A/V_D faible.

On pourrait pondérer l'interprétation des résultats en fonction de l'ensemble limité des effectifs et des paramètres génétiques utilisés. L'analyse de la question, qui englobe l'information sur les demi-germains dans la sélection d'un critère à la fois ou la sélection combinée (d'indices) mérite d'être faite.

WHAT IS A SAFE NUMBER OF CLONES PER PLANTATION?

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ABSTRACT

This paper discusses the following concepts: that a forest plantation is at risk from a great variety of possible physical and biotic events, many of which cannot be anticipated, and each has its own different probability distribution of damaging a particular population of trees; that management, through its decisions on planting density, thinning schedule, and salvage operations, can influence the amount of damage or mortality that a plantation can sustain and still be managed economically; and, that cross-adaptation of a short-generation pest may be more likely among many clones than among few unrelated clones. Some of the (perhaps unexpected) conclusions from analyses employing these concepts are: that monoclonal plantations are frequently the worst strategy, and are rarely or never the best; that mixtures of a large number of clones are as safe as seedling plantations; and, if planting density and subsequent silviculture allow damage or mortality at levels comfortably above reasonable expected risk, then mixtures of modest numbers (7-25) of clones appear to provide a robust and perhaps optimum strategy.

RÉSUMÉ

La communication traite des notions suivantes: une plantation forestière est exposée à une large gamme de phénomènes physiques et biologiques, dont beaucoup sont imprévisibles et possèdent chacun leur propre distribution de probabilité d'endommager une population donnée d'arbres; l'aménagement, par la plantation à une densité donnée, l'ordonnancement des éclaircies et les récupérations de bois, peut infleur sur le degré de mortalité ou de dégâts qu'une plantation peut subir sans perte de rentabilité; l'adaptation croisée d'un ravageur à génération courte peut être plus probable parmi de nombreux clones que parmi un nombre restreint de clones non apparentés. Certaines des conclusions (peut-être imprévues) des analyses fondées sur ces prémisses sont les suivantes: les plantations monoclonales sont fréquemment la meilleure stratégie; les mélanges de deux ou trois clones sont souvent la pire stratégie; et ils sont rarement ou jamais la meilleure; les mélanges d'un grande nombre de clones sont aussi sûrs que les plantations à partir de semis; et, si la densité de la plantation et les soins sylvicoles

ultérieurs permettent des dégâts ou une mortalité d'un taux confortablement supérieur au risque raisonable prévu, alors des mélanges de nombres modestes (7 à 25) de clones semblent constituer une stratégie sensée et peut-être optimale.

A THEORETICAL AND EMPIRICAL DISCUSSION OF SELECTION IN CLONAL FORESTRY

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ABSTRACT

A discussion is given of selection of clones for a propagation population assuming that the main selection criterion is height growth, but taking into consideration that a portion of the clones will have to be culled due to plagiotropic growth. The effect of this is a reduction of the population size and the selection intensity. The selection is assumed to be performed at two stages: an initial selection among seedlings and a selection of superior clones in a clonal test. Family information is important at the first step, particularly if the heritability is low, but the selection efficiency can also be improved by choosing the proper experimental design. The allocation of selection intensities to the two steps is discussed, and it is found that a selection of 5% of the original population at the first step seems to maximize the selection response. This percentage, however, depends both on the heritability and on the number of clones in the final population.

The traits measured in a young experiment are different from, but genetically correlated to the final traits. It is shown that predictions of genetic gain are far too high when neither this fact nor the loss due to plagiotropic growth are taken into account.

The expected selection response from bulk-propagation of superior full-sib families is compared to the response from a realistic clonal selection model.

Results from clonal experiments with Norway spruce are presented and discussed.

RÉSUMÉ

On discute de la sélection des clones pour obtenir une population destinée à la propagation, en supposant que le principal critère est la croissance en hauteur, mais en tenant compte du fait qu'une fraction des clones devra être mise au rebut à cause d'une croissance plagiotrope. Il s'ensuit une réduction de la population et de l'intensité de la sélection. On suppose que celle-ci se fait en deux étapes: sélection initiale parmi les semis, puis sélection des clones supérieurs par un test clonal. Il est important, dans la première étape, de disposer de renseignements sur les familles, notamment si l'héritabilité est faible, mais on peut aussi augmenter l'efficacité de la sélection en choisissant

le plan approprié d'expériences. L'intensité de sélection à exercer à chaque étape fait l'objet d'une discussion, et on constate qu'une sélection de 5 % de la population d'origine, à la première étape, semble maximiser la réponse à la sélection. Toutefois ce taux dépend à la fois de l'héritabilité et du nombre de clones dans la population finale.

Les traits mesurés au début de l'expérience diffèrent des traits finals, mais leur sont génétiquement corrélés. On montre que la prédiction du gain génétique est de loin trop optimiste lorsqu'on ne tient compte ni de ce fait ni des pertes dues à la croissance plagiotrope.

La réponse prévue à la sélection suite à la propagation, par la méthode bulk, de familles supérieures de pleins germains est comparée à celle que donne un modèle réaliste de sélection clonale.

Les résultats de tests clonaux avec l'épinette commune sont présentés et font l'objet d'une discussion.

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MARCH, 1985

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